AMERICAN CHEMICAL SOCIETY (ACS)

AFRICA REGIONAL CONFERENCE ON GREEN AND SUSTAINABLE CHEMISTRY

(Jointly organised by ACS Chapters in Egypt, Ghana and Nigeria)

BOOK OF PROCEEDINGS

Theme:

Green Chemistry Innovations for Sustainable Development

VENUE: Sheraton Hotel, Lagos, Nigeria - May 5-9, 2024

Featuring:

9th Annual Symposium of ACS Nigeria,

THEMED: The Role of Green Chemistry in Achieving Agenda 2063

ACS Chapter
Nigeria

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UENR University of Energy and Natural Resources

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Antimicrobial Property of *Dalbergia latifolia* Silver Nanoparticle

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ABSTRACT

The biosynthesis of nanoparticles is an ecological substitute to the chemical route which is accompanied by the discharge of harmful byproducts. Methanol extract of *Dalbergia latifolia* was used to prepare metallic silver nanoparticles (AgNPs). Optimization of the reduction was done at different operational parameters and measured using a UV-Vis Spectrophotometer. Characterization was done using spectroscopic techniques (UV, X-ray Diffraction and FTIR) and Scanning Electron Microscope. The antimicrobial efficacy of the AgNPs was determined using pathogenic microorganisms. The study, showed that the rate of production of the AgNPs increased proportionally to the time of reaction while the optimum ratio of plant extract to $AgNO_3$ solution was 1:2. The nanoparticles demonstrated activity against tested pathogens.

KEYWORDS: Silver nanoparticles, Biosynthesis, *Dalbergia latifolia*, antimicrobial activity.

1. INTRODUCTION

Metallic nanoparticles are nanomaterials with structure components of size dimensions less than 100 nanometers[1]. The high surface area to volume ratio in nanoparticles permits interaction with other particles easily[2,3] which encourages easy diffusion. In recent years, there has been increase research attention on nanoparticles owing to their outstanding qualities which have unlocked many new pathways in nanotechnology. Metal nanoparticles' optical properties are an important factor because of the localized surface Plasmon which resonance frequency within the visible region [4-6] Nanoparticle synthesis is in three major ways, namely, physical, chemical and green synthesis. Chemical reduction of metal lacks precise control over the size distribution of the colloidal particles [7] coupled with the production of damaging by-products.

Green synthesis which combines plant extracts and microorganisms is acceptable method because of its low cost and less time-consuming and also does not produce any toxic chemicals [8,9]. Applications of nanoparticles include diagnosis and management of human diseases, drugs, and fluorescent biological labels, bio-detection of pathogens[10], gene delivery agents, tissue engineering[11,12], tumour destruction[13] and dietary supplements for delivering biologically active substances. Nanoparticles are used in agriculture, cosmetics, the environment, food, home appliances, medicine, sport and fitness, and many other industrial sectors[14].
Metallic nanomaterials of Mg, Cu, Pt, Ti, Zn, Ag and Au and their corresponding oxides and sulfides have been synthesized but Ag nanoparticles have demonstrated higher efficacy against bacteria, viruses and other micro-organisms[15-18].

Dalbergia latifolia Roxb. (Bombay Blackwood or Indian rosewood) is a member of the family Fabaceae. Ethnobotanical usage includes treatment for body pain, leprosy, diarrhoea and obesity; additionally, the plant is adapted as bitter tonic, anthelmintic and for stomach ache. The bark contains tannins which is used to treat diarrhoea, worms, indigestion, and leprosy[19,20]. Antibiotic resistance is a major challenge facing infectious disease management. In the present study, the agar well diffusion technique was used to determine the antimicrobial properties of *D. latifolia* nanoparticles against ten human pathogens.

2. MATERIALS AND METHODS

2.1. Extract preparation and synthesis of AgNPs

Dalbergia latifolia leaves were obtained from the University of Ibadan, Nigeria. Identification was done at the Forest Research Institute Herbarium, Ibadan. 70 g of dried ground leaves were boiled in 40% ethanol to obtain the leaf extract. A mixture of plant extract and aqueous solution of silver nitrate was heated on a hotplate at 70^{o}C for 30 minutes. The production of silver nanoparticles was established by spectrophotometric methods.

2.2. Optimization of bioreduction Process

Synthesis of AgNPs were carried out at different operational parameters such as (i) concentration of $AgNO_3$ (1, 2, 3mM), (ii) time (0-60mins), (iii) volume ratio of plant extract to $AgNO_3$ and (iv) pH to determine their effects on the yield and properties of the synthesized nanoparticle.

2.3. Characterization of synthesized nanoparticles

UV/Vis spectral analysis was done using the Lambda 25 UV/Vis Spectrometer scanned from 300 to 900 nm. FT Infra-red spectrometer (Perkin-Elmer LS-55- Luminescence spectrometer) was used to determine the different functional groups present. The structure of synthesized silver nanoparticles was determined by X-ray diffraction spectroscopy (Rigaku D/ Max-IIIC) while the shapes and size were studied using the scanning electron microscope (JEOL JSM -7600F).

2.4. Antimicrobial assay

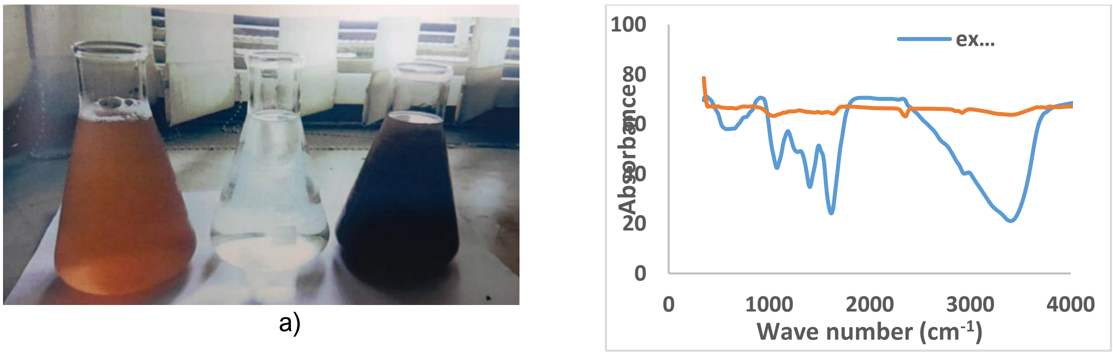
Ten pathogenic organisms which include six bacteria *Pseudomonas aeruginosa, Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumonia, Salmonella typhi, Escherichia coli* and four fungi *Candida albicans*, *Penicillium notatum, Rhizobium stolomite* and *Aspergillus niger* were used in the antimicrobial assay.

3. RESULTS AND DISCUSSION

3.1 Synthesis of Ag Nanoparticles

The colour intensity of the plant extracts increased (chocolate brown) with time (Figure 1a). This is due to the excitation of surface plasmon resonance (SPR). The green synthesized nanoparticles showed a maximum peak at 450 nm which is characteristic of the SPR band for metallic silver nanoparticles. Generally the absorption peak shows around 412–470 nm [21,22] .

The effect of various operational parameters showed that the excellent surface Plasmon resonance at a ratio of 1:1 at a concentation of 10^{-1} M for optimization. The best reaction time for optimization is 60 minutes while the effect of change in pH of the reaction time on formation of AgNPs is optimized in acidic conditions (Figures 2a-d).

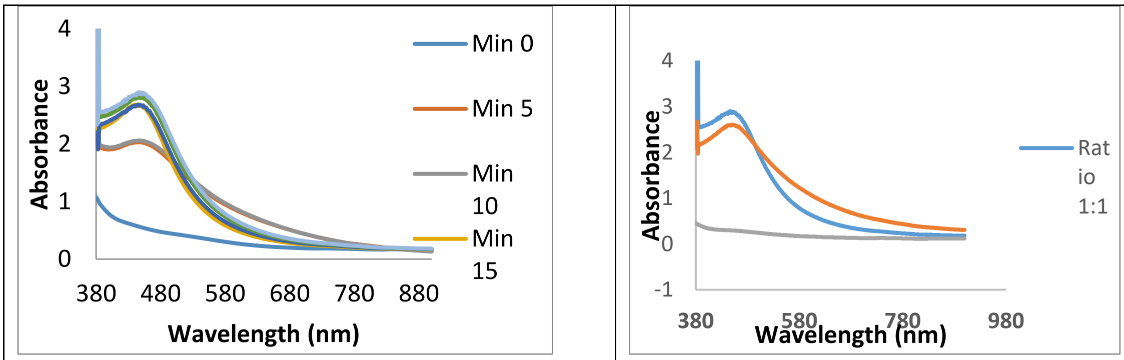


a)

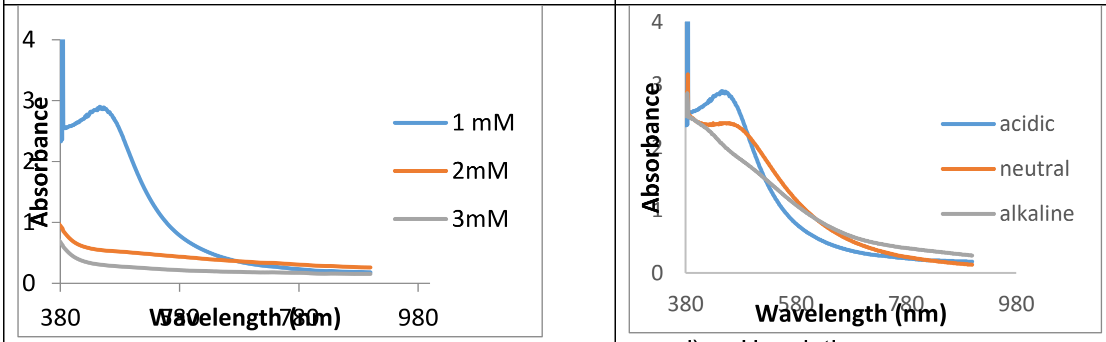
b)

Figure 1a: Solutions of leaf extract, silver nitrate, and silver nanoparticles; 1b: Graph of FTIR analysis on TD extract and AgNPs

FTIR spectra of the *D. latifolia* -AgNPS (Figure 1b) showed peaks around 1400, 1040, 3474,1700, cm-^{1} assigned to C-C, C–O–H, C=O and O-H, groups absorption bands as the possible stabilizing and capping groups[23]. The XRD result (Figure 3) confirmed the elemental silver signal. It showed characteristic peaks corresponding to metallic silver, confirming the successful synthesis of AgNPs. Energy peak at 3.0-3.8 KeV were consistent with known diffraction patterns for silver, indicating the purity and crystalline nature of the nanoparticles. SEM revealed the morphology showing the formation of uniformly distributed quasi spherical shape AgNPs (Figure 4).



a) Effect of Time b) Ratio



c) concentration d) pH variation

Figure 2a-d: Graphs of the effect of Varying Operational parameters in the formation of Silver Nanoparticles

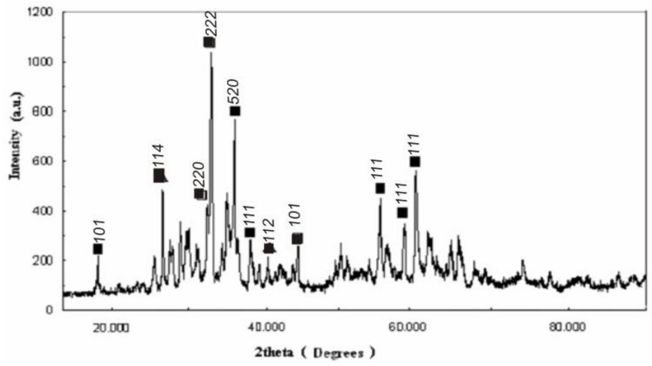


Figure 3: XRD peaks for AgNPs from *Dalbergia latifolia*

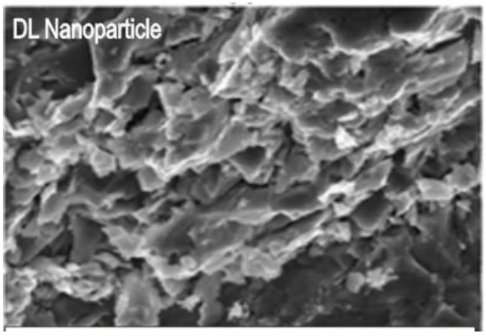


Figure 4: SEM image of *Dalbergia Latifolia* AgNPs

3.2. Antimicrobial studies

D. latifolia nanosilver showed concentration dependent zone of inhibition against all the tested microorganism. Of all the bacteria screened the gram negative, *P. aeruginosa* and *E. coli* were the most susceptible organisms while the highest activity was demonstrated against *A. niger* for fungi (Table 1). This could be because gram negative pathogens mostly contain only one single peptidoglycan coat, thus making the permeation of Ag^+ ions into the cytoplasm easy therefore causes cell lysis [24]. In all assays, the synthesized AgNPs demonstrated lower activities than the reference drugs.

Table 1: Zones of Inhibition of Green-synthesized *D. latifolia* AgNPs against Strains of microorganisms

	Zones of inhibition				
Organisms/ concentration	100 mg/mL	50 mg/mL	25 mg/mL	12.5mg/mL	6.25 mg/mL
S. aureus	20	18	16	14	10
E. coli	22	18	16	14	10
B. substilis	18	16	14	10	-
P. aeruginosa	22	18	16	14	10
K. pneumonia	18	16	14	12	10
S. typhi	16	14	12	10	-
C. albicans	18	16	14	10	-
A. niger	18	16	14	12	10
P. notatum	16	14	12	10	-
R. stolonifer	16	14	12	10	-

*Gentamicin= 38.0 mm; Tioconazole= 26.0 mm, Methanol= no inhibition

4. CONCLUSION

Green-synthesized AgNPs from *D. latifolia* exhibit promising antimicrobial properties, particularly against gram-negative bacteria and fungi. This method offers a sustainable alternative to chemical synthesis, reducing environmental impact**.** Furthermore, *D. latifolia* AgNPs could be used in combination therapies to enhance antimicrobial efficacy.

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Pd(II) Inhibition of Glucose Oxidation by $KMnO_4$ in Alkaline Medium: A Kinetic Study

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ABSTRACT

The kinetics of $PdCl_2$ inhibition of glucose oxidation by Potassium Permanganate in alkaline medium was investigated at λmax 525 nm. The oxidation reaction followed pseudo-first -order kinetics: $[\text{glucose}]_o > [KMnO_4]_o$ using a double beam Schmadzu-1800 UV/Visible spectrophotometer. A decrease in absorbance values of reaction mixtures with time were obtained and evaluated. The results showed zero order dependence to $[KMnO_4]$, first order dependence each to [glucose] and $[OH^-]$, inverse unity order to $[Pd^{II}]$ and no ionic strength effect was observed. Activated parameters for $\Delta H^\#$, $\Delta S^\#$ and $\Delta G^\#$ were 29.78 $kJmol^{-1}$, -0.21 $kJK^{-1}mol^{-1}$ and 92.36 kJ mol^{-1}, respectively. The Ardon equation revealed the formation of 1:1 and 1:2 Pd(II)-glucose complexes at low and high concentrations of glucose, respectively.

KEYWORDS: Palladium(II) Chloride, glucose, potassium permanganate, inhibition.

1. INTRODUCTION

Catalytic effects of metals on the oxidation of sugars have not received much attention. However, palladium (II) inhibition of sugar oxidation in hexacyanoferrate (III) has been reported.[1] Ru (III)-catalyzed oxidation of sugars in acidic and alkaline media was also investigated.[2] Furthermore, Ir (III) catalysis of sugar oxidation has received great attention.[3,4] The oxidation of some aldoses and amino sugars by potassium bromate in hydrochloric acid medium has been reported to proceed via the intermediate formation of bromate esters followed by the breaking down of the esters to yield products, and hydrogen ions catalyzed the reaction.[5] Oxidation of glucose and Arabinose mixtures over Au/Al_2O_3 has been reported.[6] Mechanisms of sugar oxidation in alkaline and acidic media has received great attention.[7,8,9] However, the mechanism of the catalytic effect of transition metals on sugar oxidation needs serious appraisal which prompted this research. This research seek to investigate the mechanism of Pd(II) inhibition of glucose oxidation by Potassium Permanganate in alkaline medium.

2. MATERIALS AND METHODS

2.1. Materials

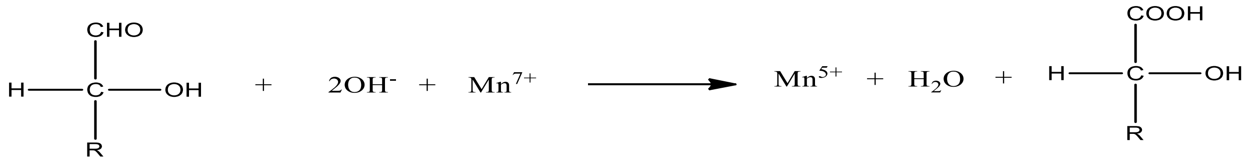
D-glucose (reductant, Merck, Germany) and $KMnO_4$ (oxidant, Merck, India) were used. All other reagents were of analytical grade. Distilled water was used as solvent.

2.2. Kinetic runs

Each kinetic run was performed under pseudo-first-order kinetics using ≥ tenfold excess [glucose] over $[KMnO_4]$. A mixture containing the requisite amount of all the reactants (except potassium permanganate) was thermally equilibrated at the desired temperature and rapidly added to a known amount of the oxidant solution (pre-equilibrated at the same temperature). Decrease in absorbance of manganese (VII) with time at 525 nm using a double-beam Schmadzu-1800 UV/visible spectrophotometer equipped with a thermo-regulated cell compartment and spectral data processing facilities was monitored. The pseudo-first-order constants (k_{obs}) were obtained from the slope of log (absorbance) versus time plots.

2.3. Stoichiometry

The reaction mixtures of known excesses of [glucose] over [$KMnO_4$] were kept in the presence of sodium hydroxide at 298 K for 3 days. Estimated unreacted [$KMnO_4$] revealed that one mole of glucose consumed one mole of potassium permanganate.



2.4. Pd (II) Complexes of Sugar

In this kinetic study, the absorbance of solutions at fixed [Pd(II)] 1.20 x 10^{-3} M and [NaOH] 1 x 10^{-3} M and varying 5 x 10^{-4} M $\leq$ [glucose] $\leq$ 1.30 X 10^{-2} M were taken at 400 nm (λ_{max} of the complex) and change in absorbance with [glucose] is shown in Figure 1.

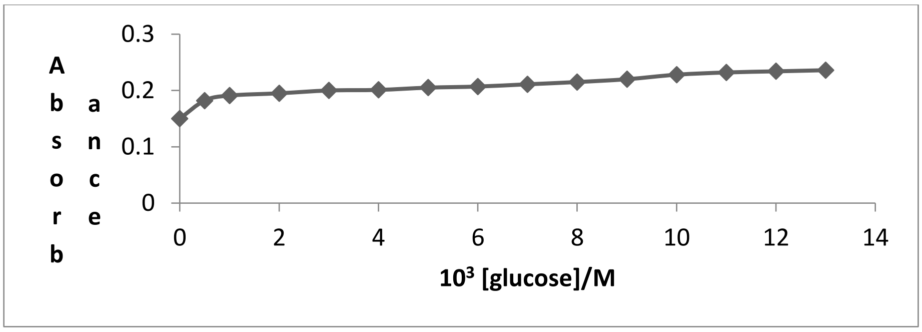


Figure 1. A plot of Absorbance vs [glucose]

From general equilibrium equation[10]

$$M + nS \rightleftharpoons M\,Sn \qquad (1)$$

$$\frac{1}{\Delta A} = \frac{1}{[S]^n}\,\frac{1}{\Delta E^{[M]}{}_{Total}K} + \frac{1}{\Delta E^{[M]}{}_{Total}} \qquad (2)$$

Herein, the amount of glucose tied up in the complex is negligible in comparison to the initial concentration of S. Therefore, a plot of $\frac{1}{\Delta A}$ Vs $\frac{1}{[S]}$ gave a straight line with intercept suggesting 1:1 complex formation between M and S at low concentrations of glucose.

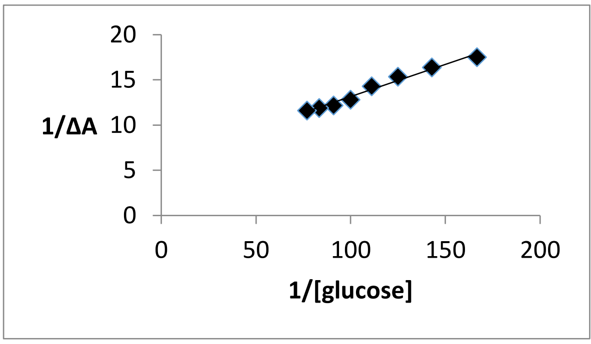


Figure 2. Plot of $\frac{1}{\Delta A}$ Vs $\frac{1}{[S]}$

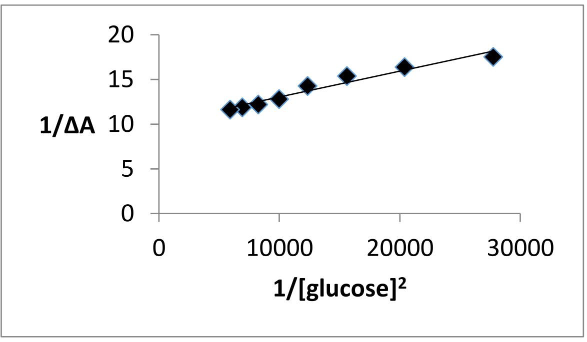


Figure 3. Plot of $\frac{1}{\Delta A}$ vs $\frac{1}{[S]^2}$

Furthermore, a plot of $\frac{1}{\Delta A}$ vs $\frac{1}{[S]^2}$ gave a straight line with an intercept, suggesting a 1:2 metal-sugar complex formation at higher glucose concentration and the apparent equilibrium constant (K) obtained from the intercept and slope was 3.50.

3. RESULTS AND DISCUSSION

3.1. Kinetic Results

The kinetic results showed relatively constant k_{obs} with [$KMnO_4$], indicating no dependence on the oxidant concentrations. However, the observed rate constant increased with increase in [glucose] as shown in Figure 4.

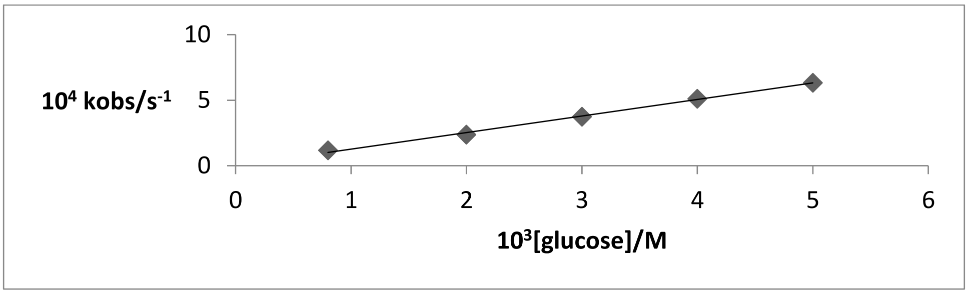


Figure 4. Plot of k_{obs} versus [glucose]

The order of reaction with respect to [glucose] obtained from log k_{obs} versus log [glucose] gave one, suggesting a first order kinetics with respect to [glucose]. There was an increase in pseudo first order rate constant with increase in [OH^-] as shown in figure 5, and the order with respect to [OH^-] was obtained to be one.

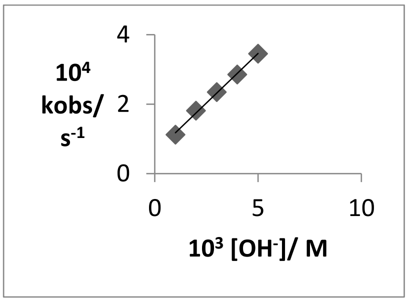


Figure 5. Plot of k_{obs} versus [OH^-]

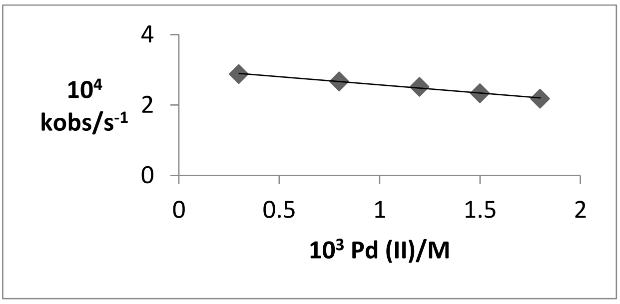


Figure 6. Plot of k_{obs} versus [Pd(II)]

A plot of k_{obs} versus [Pd(II)] was linear with negative slope with an intercept as shown in figure 6.

Activation parameters were obtained from Erying's plot from temperature dependent study as shown in Table 1.

$$\mathrm{In}\left(\frac{k}{T}\right) = \frac{-\Delta H^{\#}}{RT} + \mathrm{In}\left(\frac{K^{/}}{h}\right) + \left(\frac{\Delta S^{\#}}{R}\right)$$

$$\text{where}\quad \mathrm{In}\left(\frac{K^{/}}{h}\right) = 23.76$$

$$\Delta G^{\#} = \Delta H^{\#} - T\Delta S^{\#}$$

k = rate constant; T= Temperature; $\Delta H^{\#}$ = Enthalpy of Activation; $\Delta S^{\#}$ = Entropy of Activation; $\Delta G^{\#}$ = Free Gibb's Energy of Activation; R = Molar gas constant; $k^{/}$ = Boltzmann constant; h= Planck's constant.

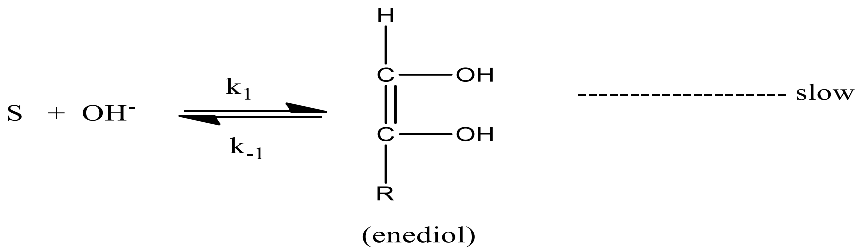
Table 1. Activation parameters

Substrate	$\Delta H^{\#}$ /kJ mol^{-1}	$-\Delta S^{\#}$ /kJ K^{-1} mol^{-1}	$\Delta G^{\#}$ /kJ mol^{-1}
Glucose	29.78	0.21	92.36

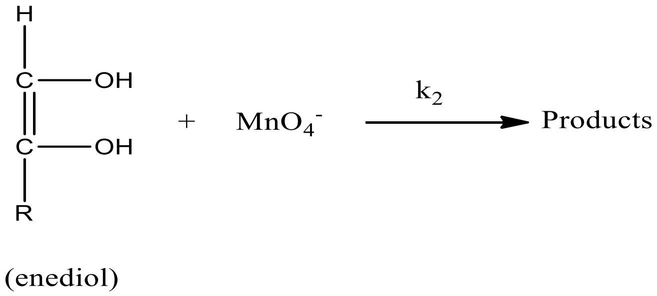
$[KMnO_4]$ 3 x 10^{-5} M, [glucose] 3 x 10^{-3} M, $[OH^-]$ 1 x 10^{-3} M, [Pd(II)] 1.20 x 10^{-3} M, μ 0.05 M

3.2. Discussion

The un-catalyzed oxidation of sugars by alkaline permanganate showed first order dependent each to $[OH^-]$ and [glucose] and zero order with respect to $[KMnO_4]$.[8] Furthermore, this study revealed no change in k_{obs} with increase in ionic strength of the reaction mixtures. Consequently, a plot of log k_{obs} versus √I gave straight line with Z_AZ_B equals to zero, suggesting that ionic strength did not affect the oxidation reaction, thereby revealing the presence of a neutral molecule in the rate determining step. The mechanism involved the formation of an intermediate enediol of sugars in alkaline medium which subsequently react with potassium permanganate to give the product and palladium(II) chloride formed complexes with glucose in the course of the reaction.



(3)



(4)

$$S + Pd^{2+} \overset{K_3}{\rightleftharpoons} X \qquad (5)$$

$$X + S \overset{K_4}{\rightleftharpoons} Y \qquad (6)$$

Based on the kinetic results obtained in this research, the mechanism reported herein followed that which was earlier reported.[1] The complexes are represented as (X) and (Y). In this study, the formation of 1:1 and1:2 complexes of Pd (II) chloride with glucose at lower and higher concentrations of glucose respectively were confirmed. The decrease in the pseudo first order rate constants with increase in Pd (II) on the oxidation reaction shows that palladium complex resisted the oxidation reaction.

The mechanism of inhibition was proposed as follows:

$$\frac{-d[MnO_4^-]}{dt} = \frac{k_1k_2[S][OH^-][MnO_4^-]}{k_{-1} + k_2[MnO_4^-]} \qquad (7)$$

$$k_2[MnO_4^-] \gg k_{-1}$$

$$\frac{-\mathrm{d}[\mathrm{MnO_4^-}]}{\mathrm{dt}} = \mathrm{k_1[S][OH^-]} \qquad (8)$$

$$[S]_T = [S] + [X] + [Y] \qquad (9)$$

$$[X] = K_3[S][Pd^{II}]$$

$$[Y] = K_3K_4[S]^2[Pd^{II}]$$

$$[S]_T = [S] + K_4[S][Pd^{II}] + K_3K_4[S]^2[Pd^{II}]$$

$$[S]_T = [S](1 + K_3[Pd^{II}] + K_3K_4[S][Pd^{II}])$$

$$[S] = \frac{[S]_T}{1 + K_3[Pd^{II}] + K_3K_4[S][Pd^{II}]}$$

Substituting in equation (8)

$$\frac{-\mathrm{d}[\mathrm{MnO_4^-}]}{\mathrm{dt}} = \frac{\mathrm{k_1[S]_T[OH^-]}}{1 + K_3[Pd^{II}] + K_3K_4[S][Pd^{II}]} \qquad (10)$$

Rate law (10) reveals first order and zero order dependence of the rate with respect to $[OH^-]$ and $[KMnO_4]$ respectively which is in consonance with the experimental results. It also showed the inhibition effect of Pd(II) on the oxidation reaction. Furthermore, at low [glucose], when 1:1 complex is predominant $K_4 = 0$. Therefore, the rate law can be expressed as:

$$\frac{-\mathrm{d}[\mathrm{MnO_4^-}]}{\mathrm{dt}} = \frac{\mathrm{K_1[S]_T[OH^-]}}{1 + K_3[Pd^{II}]} \qquad (11)$$

4. CONCLUSION

Pd(II) inhibited the oxidation of glucose by potassium permanganate in alkaline medium. 1:1 and 1:2 Pd(II)-glucose complexes were obtained via Ardon plots at lower and higher concentrations of glucose, respectively. The reaction was zero order to $[KMnO_4]$, first order each to [glucose] and $[OH^-]$ and inverse first order to [Pd(II)] and an independent ionic strength effect was observed.

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The Use of Eel Fish as a Bioindicator for Heavy Metals Pollution of Surface Water: A Case Study of Ibeno Beach, Akwa Ibom State

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ABSTRACT

The pollution of the aquatic environment with heavy metals is a worldwide problem. Given their hazardous nature, lack of biodegradability, and cumulative tendencies, these substances are deemed hazardous environmental contaminants that require monitoring. One of the effective ways is by analyzing their accumulation in water, sediments, and biota. This study evaluates the levels of some heavy metals in water samples from Ibeno Beach using the Eel fish *(Anguilliformes)*. Water samples were taken from four different sampling points 400 meters apart. The fish samples from the beach were homogenized and digested using an oxi-acidic mixture. Both water and fish samples were analyzed using Graphite Furnace Atomic Absorption Spectrophotometer (GF-AAS). Mean concentration of the five heavy metals in this study in the water samples analyzed gave the following results of 0.236±0.067, 0.041±0.014, 2.231±0.430 and 0.056mg/L for Cr, Cd, Fe and Ni respectively. Pb was not detected. The mean concentrations obtained for the fish samples were as follows; 0.294±0.027, 0.060±0.005,1.371±0.061, Ni 0.044 and 0.232±0.020 mg/L for Cr, Cd, Fe, Ni and Pb respectively. The order of the metals occurrence in the beach water is Fe > Cr > Ni > Cd > Pb and for the Eel fish samples, Fe > Cr > Pb > Cd > Ni. These orders show close similarity indicating that the Eel fish can be used as a bioindicator for the assessment of heavy metal pollution of surface water such as the Ibeno Beach.

KEYWORDS: Eel fish, Ibeno Beach, Biota, Bioaccumulation.

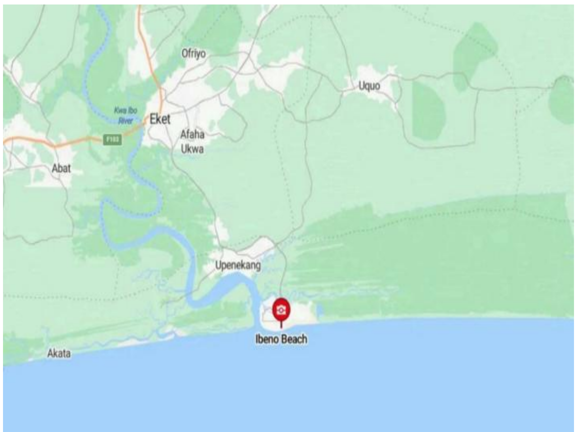
1. INTRODUCTION

Environments along the sea coast related to land are mostly affected by pollution emanating from anthropogenic activities and the discharge of effluents. Potentially toxic elements can affect the aquatic biota, posing health hazards to fish consumers, through the food chain.[1,2] Given their hazardous nature, lack of biodegradability, and cumulative tendencies, these substances are deemed hazardous environmental contaminants that require monitoring. The majority of metals with a geochemical origin are found in less accessible or unreachable forms. Living organisms are affected by both essential and non-essential components in an ecotoxicological way.[3,4] Although metabolic activities in organisms necessitates the presence of some metals like iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), and other trace elements, potentially toxic metals such as lead (Pb), nickel (Ni), cadmium (Cd), mercury (Hg), chromium (Cr), and arsenic (As) are marked as hazard due to their toxic nature. Generally, an analysis of the accumulation of these metals in water, sediments, and related biota is used to track their levels in the aquatic environment. Accumulated metal in water are found considerably lower when compared to sediment and biota. Macroalgae has been used as bio-monitors of heavy metal contamination in a tropical estuary in Malaysia.[5] The use of fish and shrimps as an environmental indicator for heavy metal pollution in water and sediments was reported[6]. Fish are widely regarded as a significant bio-indicator of the aquatic environment. A few environmental factors influence the amount of bioaccumulated heavy metals in fish bodies.[7] Eel Fish is an aquatic organism that is rich in Omega 3 and it is of great economic importance.[8] Consuming Eel supports brain development, helps maintain good eye health, strengthens the bones, prevents stroke and anemia.[9] It has the ability to aid skin cells regeneration thereby reducing wrinkles and improving the look of the skin, hair and nails; due to its high collagen content.[10] The presence of potentially toxic elements in Eket River will affect the aquatic life, and individuals consuming any fish or seafood. This study is therefore aimed at assessing the presence and levels of these elements using the Eel fish which is commonly found in this river and around its environs.

2. MATERIALS AND METHODS

2.1. Study Area

The sample area,Ibeno Beach is in Akwa Ibom State, Nigeria. It is located at 4.568693^{0}N and 7.976396^{0}E and occupies a vast coastal area of over 1, 200km^2.



Picture 1: Map showing Ibeno Beach **Picture 2:** A section of Ibeno Beach

The occurrence of heavy metals in this surface water is mostly from anthropogenic sources. The boats are usually washed at the beaches where metal scraps and particles are discharged into the water making them available to the aquatic biota. The runoffs from farms and settlements around the beach contribute to the levels of heavy metals in the marine ecosystem.

2.2. Sample collection

Water samples were collected from the activity side of the beach where the landing platform is in plastic bottles from four different points away from the landing point. The Eel fish *(Anguilliformes*) was collected with the help of local fishermen in clean sterile poly bags.

2.3. Physicochemical analysis

The pH and temperature were determined *in situ*.

2.4. Digestion of Fish samples

The fish samples were cut up in small pieces and placed in the oven to dry at a temperature of 120^{o}C until crisp. They were crushed using a porcelain mortar and pestle in the laboratory. 1g of the homogenized crushed fish sample was weighed out in triplicates into three different beakers and 20 ml of the oxi-acidic digestion mixture of nitric acid and perchloric acid (4:1) were added to each beaker. The samples were heated in the fume chamber until clear solution was obtained and the contents of the beakers were allowed to cool, then filtered using Whatman filter papers (No 40) into 50ml volumetric flasks. These were made up to the mark with deionized water. The water and fish samples were taken to the laboratory for analysis using a graphite furnace Atomic Absorption spectrometer (GF-AAS) with air-acetylene flame.

3. RESULTS AND DISCUSSION

The results obtained from the four sampling points for the water samples are presented in Table 1. The mean concentrations for Cr, Cd, Fe and Ni are 0.236 ± 0.067 mg/L, 0.041 ± 0.014 mg/L, 2.231 ± 0.430 mg/L and 0.056 ± 0 mg/L respectively. Pb was not detected. Fe concentrations were much higher than the other metals. This can be attributed to the human activities including ship and boat traffic at this beach. The wear and tear from them can contribute to the natural quantities of Fe present in the water.

Cr in its VI oxidation state is of no benefit. It causes skin ulceration, liver and kidney damage, severe cardiovascular, gastrointestinal and respiratory problems.[11] Ni enters water bodies through weathering of soils and rocks,[12] leaching and discharge of Ni-containing effluents from nearby industries.[13] This accounts for the high levels of Ni found in this study which is above the maximum acceptable limit of 0.02 mg/L by WHO. However, the result is lower than that reported by Luczynska et al.[14] in a similar study. Ni is essential in the synthesis of red blood cells in trace quantities. However, in high doses, it causes damage to the liver and heart, reduction in cell growth, CNS dysfunction and cancer.[15, 16] Cd is not essential in biological systems. It causes renal dysfunction, lung and prostate cancer, osteomalacia [17] and is classified by IARC as group carcinogen even at low concentrations. From the result obtained the heavy metal concentration in the water sample follows the order Fe >Cr > Ni > Cd > Pb. All the metal analyzed in the water samples were above WHO[21] acceptable limits.

Table 1: Heavy metal concentration for water samples

Water sample	Cr (mg/L)	Cd (mg/L)	Fe (mg/L)	Pb (mg/L)	Ni (mg/L)
Point 1	0.210	0.052	1.816	ND	0.056
Point 2	0.196	0.020	2.701	ND	ND
Point 3	0.200	0.042	2.486	ND	ND
Point 4	0.336	0.049	1.920	ND	ND
Mean	0.236	0.041	2.231	-	0.056
Std Dev.	0.067	0.014	0.430	-	0
WHO	0.1	0.003	0.5	0.015	0.02

The results from the eel fish samples collected from the four sampling points are presented in Table 2. From these results, Fe has the highest concentration ranging from 1.287 mg/kg to 1.428 mg/kg with an average of 1.371 ± 0.061 mg/kg. This value is lower than that obtained from the water samples. Fish samples from Point 2 has the highest concentration (1.428 mg/kg). Fe is an essential element in human metabolism and other living things. It is a major component of heam. High levels of Fe in the human body leads to iron overload and this is not acceptable. The average concentration of Cr is 0.294 ± 0.027 mg/kg which is higher by 0.068 mg/kg than that obtained from the water samples. Sampling Point 3 recorded the highest (0.320 mg/kg) followed by Point 4, then Points 2 and 1. The levels of Cr in this edible fish is within FAO[22] acceptable limits. The results for Cd are slightly higher than that obtained from the water samples by 0.020 mg/kg. According to Aytekin et al.[6] the levels of Cd and Pb in fish and shrimp can serve as a marker for the environmental levels of these metals. Pb is extremely toxic, harms hemoglobin synthesis and has been implicated in carcinogenic processes, replacing Zn in certain proteins.[17, 18] The levels of Cd and Pb were found to be within acceptable limits[22]. The average concentration is 0.232 ± 0.020 mg/kg with sampling point 3 having the highest (0.256 mg/kg) and sampling point 2 having the least (0.210 mg/kg). From these results, there is no risk of Pb poisoning from consuming the eel fish from this beach. Ni was detected from only sampling point 1and its value (0.044 mg/kg) is within the acceptable limits[22]. The heavy metal concentration in the fish samples follows the order Fe > Cd > Ni > Pb > Cr. All the heavy metal analyzed in the fish samples were found to be within acceptable limits[22] except Fe.

Table 2: Heavy Metal Concentration for Fish Samples.

Fish sample	Cr (mg/kg)	Cd (mg/kg)	Fe (mg/kg)	Pb (mg/kg)	Ni (mg/kg)
Point 1	0.258	0. 059	1.370	0.221	0.044
Point 2	0.288	0.066	1.428	0.210	ND
Point 3	0.320	0.055	1.398	0.256	ND
Point 4	0.308	0.060	1.287	0.239	ND
Mean	0.294	0.060	1.371	0.232	0.044
Std Dev.	0.027	0.005	0.061	0.020	0

FAO	2.30	0.20		0.30	5.0

Table 3 gives the chronic daily intake and hazard quotient for the metals. The daily reference dose (R_fD) is the dosage that indicates the level of exposure of a particular heavy metal in the body system of an individual without causing any harmful effect to such an individual. R_fD values (mg/kg/day) for the metals are as follows: Cr 0.0003, Cd 0.0015, Fe 0.007, Pb 0.0035, Ni 0.02.[20] HQ >1 represents an adverse non-carcinogenic effect; HQ < 1 represents permissible levels of no concern. From the HQ results, it shows that the metal concentrations are within acceptable levels and therefore there is no concern.

Table 3: Values for Chronic Daily Intake and Hazard Quotient for the metals

Metal	Mean concentration (mg/L)	CDI (mg/kg/year)	Daily CDI (mg/kg/day)	HQ $(\frac{CDI}{RfD})$
Cr	0.294 ± 0.027	0.024	6.58 E-5	0.219
Cd	0.060 ± 0.005	0.005	1.37 E-5	0.027
Fe	1.371 ± 0.061	0.113	3.10 E-4	0.044
Pb	0.232 ± 0.020	0.019	5.21 E-5	0.015
Ni	0.044 ± 0.000	0.004	1.10 E-5	0.001

4. CONCLUSION

From the HQ results, it shows that the metal concentrations are within acceptable levels and therefore there is no concern. However, care should be taken in the continuous consumption of eel from this beach. It can lead to the bioaccumulation of these potentially toxic elements, as fishes have been known to have a strong potential for accumulating pollutants including metals. The continuous monitoring of this beach and other water fronts for the presence of potentially toxic elements using the indigenous fishes that are predominantly found in such surface water is recommended.

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BOOK OF PROCEEDINGS
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Synthesis and Characterization of Mn (II) Nano Metal Complex of Trimethoprim using Sonicator

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ABSTRACT

Trimethoprim, a known antibiotic used in combination with sulfamethoxazole against several strains of bacteria, works by inhibiting the dihydrofolate reductase synthesis in bacteria. This combination therapy of trimethoprim-sulfamethoxazole has achieved outstanding antimicrobial results; however, the clinical misuse of this combination therapy has caused the development of resistant pathogens and unpleasant side effects, springing up the necessity of alternatives for increased mechanisms of antibacterial actions. Metal complexes have been reported to increase the therapeutic efficiency of drugs. Mn (II) nano metal complex of trimethoprim was synthesized by the sonication method. A change in color and reduction in melting point suggested an occurrence of complexation. Spectroscopic analyses indicated the synthesis of a metal complex. The nanosized metal complex was observed to be insoluble in hexane due to an increase in the polarity after complexing with a metal. The crystallite size of 4.04 nm was calculated using Debye-Scherrer's equation. In comparing the FTIR spectra of TMP and the Mn (II) complex, a band shift of the azomethine bond from 1633 cm^{-1} to 1595 cm^{-1} as well as the amine bond from 3317 cm^{-1} in the ligand to 3354 cm^{-1} in the complex was observed. A shift of the methylene protons from 3.533 ppm to a higher field of 1.024 ppm was observed in the proton NMR, and from 32.92 ppm to 18.39 ppm in the carbon-13 spectra indicates a complexation involving the methylene carbon. A diagonal bipyramid structure was proposed for the novel Mn (II) complex. Trimethoprim coordinated as a polydentate ligand to the Mn (II) ion.

KEYWORDS: Trimethoprim, nano metal complexes, Mn (II), sonochemistry.

1. INTRODUCTION

Trimethoprim (TMP) is a therapeutic compound with a strong potential of inhibiting the dihydrofolate reductase in bacteria. This property amplified its use especially in combination therapy with sulfamethoxazole in treatment of several infections (Asogwa *et al.,* 2024; Edozie *et al.,* 2020; Otuokere *et al.,* 2019). TMP as a compound has 1,2,3-trimethoxybenzene and pyrimidine-2,4-diamine in its structure and belonging to diaminopyrimides group of medicines (Ibrahim *et al.,* 2019, Abeer and Moamen, 2024). It has shown to have strong antimicrobial properties against both gram-positive and gram-negative strains of bacteria as well as antifungal effect on different fungi (Masoud *et al.,* 2019) but resistance could be developed by these organisms when trimethoprim is used as a single therapy (Drews, 2000, Otuokere *et al.,* 2024).

The discovery of therapeutic compounds that could inhibit the absoption of the folic acid by the bacteria has greatly increased the success in the treatment of bacterial (Otuokere, *et al.,* 2022, Otuokere *et al.,* 2020). The development of active antimicrobial compounds became one of the most relevant achievements of the twentieth century (Asogwa and Otuokere, 2024). The rampart abuse of these known antimicrobial drugs led to the development of resistant strains of bacteria (Sirajul *et al.,* 2020) making it so necessary for the discovery and administration of more effective therapeutic compounds in the form of novel compounds or structural modified drugs (Otuokere *et al.,* 2017). These modified compounds could be achieved by the coordination of the known therapeutic compounds with metal ions which has been noted in recent researches to have improved the biological effects (Otuokere *et al.,* 2020, Ugochukwu and Otuokere, 2021).

Trimethoprim has shown to be a good ligand leading to formation of several metal complexes with different metals because of the presence of three nitrogen atoms in its structure (Refat *et al.,* 2021) but also known for its poor water solubility (ElShaer *et al.,* 2012). These metal complexes of trimethoprim synthesized have been characterized to give a higher therapeutic activity than the free trimethoprim drug (Ibrahim *et al.,* 2019). This then gave the rationale for the synthesis of different metal complexes of trimethoprim. To our knowledge, there are no documented instances of sonication methods employed in the production of metal complexes of trimethoprim. Additionally, the Mn (II) nano-sized complex of TMP has not been reported in the literature.

2. MATERIALS AND METHODS

2.1. Compound and solvents used

All the salts used in this synthesis were of analytical grade obtained from Andhra Organics Limited, India.

2.2. Synthesis of the complex

The nanometal complexes were synthesized according to the methodology outlined by Abdel-Rahman *et al.* 2016. Equimolar solutions of the ligand and metal ion were obtained by dissolving 2.9032 g of trimethoprim in 100 ml of ethanol and 1.9791 g of $MnCl.4H_2O$ in 100 ml of water. These equimolar solutions were added together stirred and placed on the probe of an ultrasonic sonicator having a maximum force output of 400 W for a duration of 30 minutes. The obtained mixture obtained was filtered with a Whatman No. 1. filter paper and dried with a desiccator.

2.3. Physical and spectroscopic studies

(a). Melting Point of test compounds

The Melting points of the test compounds were evaluated with Gallenkamp apparatus.

(b). Solubility Test

The solubility profile of test compounds was investigated using solvents of variable polarity namely: hexane, ethanol, water, ethyl acetate, and dimethylsulfoxide by taking 0.1 g of each compound and dissolving in 3 ml of each solvent at 25 °C.

(c). Infrared spectroscopy

FT-IR spectra for the test compounds were obtained with the help of a Perkin Elmer Spectrum BX FT-IR spectrophotometer (4000–600 cm^{-1}).

(d). X-ray Diffraction

The Diffraction pattern for the nanosized complex was obtained with the use of Rigaku D/Max-IIIC X-ray diffractometer. The Debye-Scherrer's equation; $D=K\lambda/(\beta \cos \theta)$ where D is the crystallite size of test compound., K is the Scherrer constant = 0.98, ƛ is wavelength, β is the full width at half maximum (FWHM) was used to determine the crystallite size of the compound.

(e). Nuclear magnetic resonance

The NMR spectra for the nano-sized complex was obtained using a Nanalysis X-685 benchtop NMR which has a frequency of 600 MHz and deuterated DMSO used as solvent.

3. RESULTS AND DISCUSSION

The physical properties are presented in Table 1.

Table 1: Physical parameters

Compounds	Color	Melting Point (°C)	Percentage Yield
Trimethoprim	White	199	-
$[Mn(TMP)(H_2O)_2]$	Green	196	74

TMP and Mn (II) nanometal complex are observed to have a crystalline structure, having non-hygroscopic property and stable in air. A change of color from the white color of the ligand to green pointed to a coordination since transition metal complexes are colored (Trupti *et al.,* 2019).

3.1. Solubility

The solubility profile of the test compounds is presented in Table 2.

Table 2: The solubility profile.

Compounds	Hexane	Ethanol	Distilled water	Ethyl Acetate	DMSO
Trimethoprim	SS	S	IS	S	S
[Mn(TMP)(H_2O)$_2$]	IS	SS	SS	S	S

Key: SS- slightly soluble, IS- insoluble, S-soluble

The synthesized Mn (II) nano metal complex was soluble in ethyl acetate and DMSO and not soluble in hexane. This could be attributed to the fact that DMSO and ethyl acetate are polar aprotic solvents. These are solvents that can dissolve polar as well as non-polar substances and are also very miscible with a wide range of organic solvents and water.

The nano complex was only slightly soluble in ethanol. The solubility profile suggested that the nano metal complexes have considerable higher polarity in comparison with the ligand.

3.2. Infrared Spectroscopy

Some IR peaks of the test compounds are shown in Table 3.

Table 3: Summary of the IR peaks

Compounds	Absorption bands in cm^{-1}					
	NH_2	C-O-C	C=N	M-N	M-C	M-O
TMP	3317	1126	1633	-	-	-
[Mn(TMP)(H_2O)$_2$]	3354	1133	1595	1025	880	-

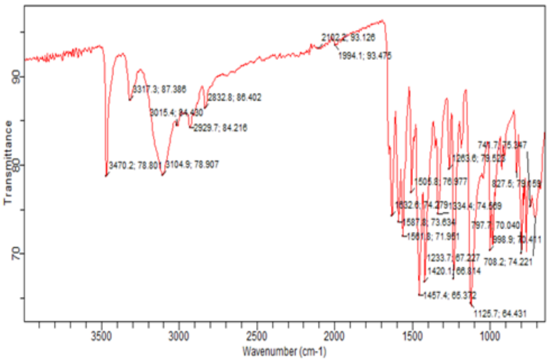

Figure 1a: IR spectrum of Trimethoprim

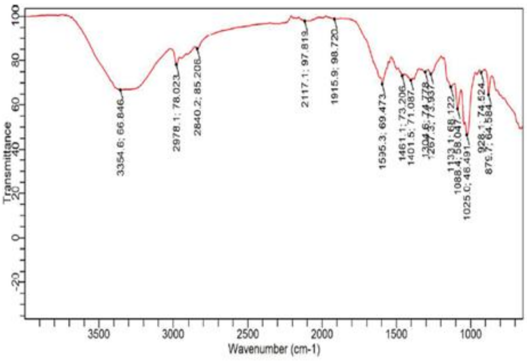

Figure 1b: IR spectrum of [Mn(TMP)(H_2O)$_2$]

The shifting of the azomethine (C=N) vibrations from 1633 cm^{-1} in the spectra of trimethoprim (Asogwa *et al.,* 2024) to 1595 cm^{-1} in the nanometal complex confirmed the coordination of the Mn (II) to the imine nitrogen atom (Aderoju *et al*., 2015). The band at 3317cm^{-1}, in the IR spectrum of trimethorpim, was assigned to NH_2 (Asogwa *et al.,* 2024). This band shifted to 3354cm^{-1} in the spectrum of the nano metal complex, this indicates that there was coordination of the metal ion to the nitrogen of the amino group without deprotonation (Beyrambadi *et al*., 2011).

3.3. XRD

The X-ray Diffraction data is presented in Table 4.

Table 4: The XRD spectra data of the novel complex

Compound	λ (Å)	2ϴ	FWHM	Crystallite size (nm)
$[Mn(TMP)(H_2O)_2]$	1.551	25.169	2.116	4.043

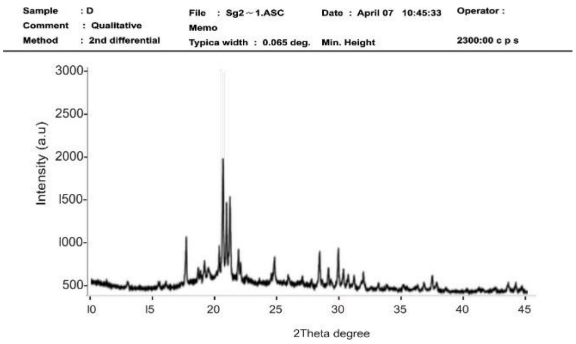

Figure 2: XRD pattern of $[Mn(TMP)(H_2O)_2]$

The XRD pattern showed that the Mn (II) nanocomplex is crystalline with a crystallite size of 4.043 nm, respectively.

3.4 NMR

The ^{1}H and ^{13}C NMR spectral data are summarized in Table 5 and Table 6 respectively .

Table 5: Summary of the ^{1}H NMR bands

Compounds	Chemical shift in ppm				
	Benzyl protons	methoxy protons	methylene protons	pyrimidine protons	H_2O protons
TMP	6.559	3.725, 3.620	3.533	7.529	-
$[Mn(TMP)(H_2O)_2]$	6.627	3.750, 3.591	1.042	7.327	3.242

Table 6: Summary of the ^{13}C NMR bands

Compounds	Chemical shift in ppm			
	CH_2	C-N	C=N	C=C
TMP	32.92	155.00	162 .00	105.00
$[Mn(TMP)(H_2O)_2]$	18.39	155.00	163.00	107.00

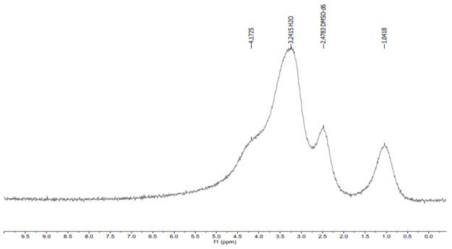

Figure 3a:^{1}H spectrum of [Mn(TMP)(H_2O)$_2$]

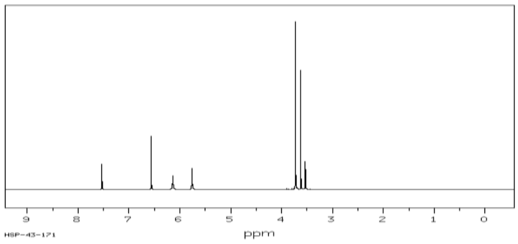

Figure 3b:^{1}H spectrum of TMP (source: spectra database for organic compounds SDB)

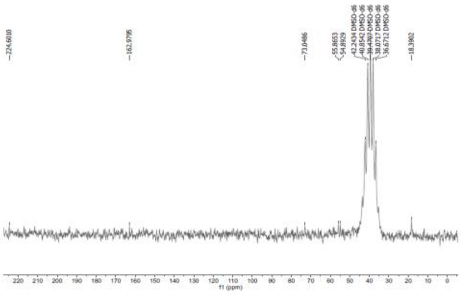

Figure 4a: ^{13}C NMR spectrum of [Mn(TMP)(H_2O)$_2$]

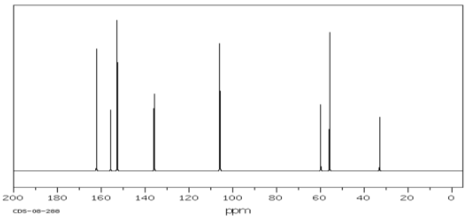

Figure 4b:^{13}C NMR spectrum of TMP (source: spectra database for organic compounds SDBS)

The proton NMR spectrum of trimethoprim has a singlet peak at 3.533 ppm, attributed to the methylene proton. This was observed at a higher chemical field in the ^{1}H NMR spectrum of the nanometal complexes, showing coordination to a metal atom (Asogwa *et al*., 2024). The protons of the NH_2 group were observed at 6.14 and 5.76 ppm in the ligand spectrum. These peaks shifted upfield in the spectra of the nanometal complexes owing to coordination with the metal ions (Otuokere *et al.,* 2022).
In the ^{13}C NMR, there was a significant shift of the methylene carbon from 32.92 ppm to 18.39 ppm attributed to the withdrawal of electrons from the carbon atom due to the coordination.

Based on the spectroscopic study, the proposed structure for the [Mn(TMP)(H_2O)$_2$]nanocomplex is:

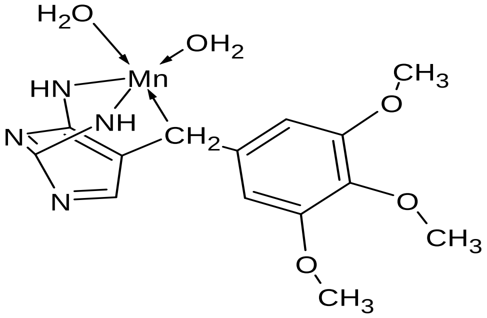

Figure 5: Proposed structure for [Mn(TMP)(H_2O)$_2$]

4. CONCLUSION

Synthesis and characterization of Mn (II) nanocomplex of TMP was achieved. The novel nanometal complex was synthesized by the use of sonication method and characterized utilizing spectroscopic analysis such as FTIR and NMR spectra and physical properties such as melting points and solubility profile. The X-ray Diffraction pattern showed the Mn (II) nanocomplex to be crystalline and having a crystallite size of 4.043 nm. A diagonal bipyramid structure was proposed for the complex showing that TMP behaved as a polydentate ligand towards the Mn (II) ion.

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BOOK OF PROCEEDINGS
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Characterization and Reinforcement of *Zea mays* Fibre in Geopolymer Composite

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ABSTRACT

For technical, economic and environmental reasons, the construction industry now widely accepts the development of composites using natural fibres and eco-friendly materials in structural concrete. The goal of this study was to create a composite made from cornstalk (*Zea mays*) fibre, an agricultural waste and geopolymer. The *Zea mays* fibre was extracted and characterized for its physical properties and chemical composition. The geopolymer precursor, meta-kaolin, was activated using an equal volume of sodium silicate and 12% sodium hydroxide. The impact of the cornstalk fibre sizes (0.425mm and 1mm) and fibre volume (1%, 2.5%, 5%, 7.5%, and 10%) on the compressive strength of the reinforced geopolymer composite were investigated. The mean values obtained for the physical and chemical properties of cornstalk fibre were 10%, 20.5%, 25.5%, 45.3 %, 11.5 %, 0.39 gcm^{-3}, 35.3% and 49.67% for moisture content, cold water solubility, hot water solubility,1 % NaOH, alcohol-benzene solubility, density, lignin content and cellulose content. The mean values obtained for compression strength of geopolymer composite reinforced with 0.425 mm corn stalk fibre ranges from 15.527 N/mm^2 – 38.80 N/mm^2 and for 1.00 mm it ranges from 18.02 N/mm^2 – 41.74 N/mm^2. The results revealed that an increase in cornstalk fibre improves the compressive strength of the geopolymer composite concrete and that the higher mechanical strength was observed with geopolymer reinforced with 1.00 mm fibre size. In conclusion, the findings show that the cornstalk fibre were suitable for reinforcement.

KEYWORDS: *Zea mays*, Composite, Geopolymer, Reinforcement, Compressive strength.

1. INTRODUCTION

The development of composites using natural fibre and more eco-friendly matrices has been prompted by the demand for energy-efficient building and industrial components. Composites exist in nature. Composites are materials that consist of two or more materials that are combined in a way that allows the materials to stay distinct and identifiable. The goal of composites is to allow the new materials to have strengths from both materials, often covering the original materials' weaknesses[1]. The two constituents of composites are matrix and reinforcement materials. The matrix adopted in this study is geopolymer made from meta-kaolinite and its activator, sodium silicate and sodium hydroxide and the reinforcement material is Corn (*Zea mays*) stalk waste fibres.

Corn (*Zea mays*) waste fibres are lignocellulosic fibres consisting of corn cob, corn husk, corn stalk and corn stover, often discarded[2, 3]. Physio-chemical properties of the lignocellulosic fibres from different sources are compared. Studies on lignocellulosic fibre-reinforced bio-plastic composites and state-of-the-art fibre-reinforced composites are reviewed[4]. The preparations of the fibres to form nano-fibril, cellulose nano-fibril and lignin-containing cellulose nano-fibril are also discussed.

Along with this, issues to improve fibre-plastic matrix compatibility through mechanical disintegration and surface modification treatment on fibre are also reported [5, 6]. Further treatment of the fibres could improve the composite properties for various applications[7]. In this study, the aim is to create a composite made from cornstalk (*Zea mays*) fibre, an agricultural waste reinforced with a geopolymer; also, to determine the functional group in *Zea mays* fibre using FTIR.

2. MATERIALS AND METHODS

2.1. Sample collection and preparation

Dry cornstalks used were collected on a farm at Saki town, Agosuru off Irawo road Agbele in Oyo State, Nigeria. The husks, leaves and inner part (pitch) were manually removed from the stalk's outer part, which was grinded into smaller particle sizes. The geopolymer precursor (kaolin) used was collected from Mile 7, Abeokuta, Ogun state, Nigeria. The kaolin was calcined at 600°C for three hours from dehydroxylation to meta-kaolinite[7]. The pure geopolymer process was done by activating the precursor with an equal volume of sodium silicate and sodium hydroxide while sodium hydroxide concentration was varied (4%, 8%, 12% and 16%). A geopolymer of 12% concentration was observed with the highest compression strength and this percentage of sodium hydroxide was adopted for the subsequent composite fabrication[8].

2.2. Determination of Physical and Chemical Properties

Moisture content, cold water solubility, hot water solubility, 1% NaOH, alcohol-benzene solubility and density were determined using ASTM standard. The lignin and cellulose content were also obtained using TAPPI and Kushner-Hoffer methods respectively[9].

2.3. Geopolymerization process and composite formation

Kaolin was calcined at 700°C for 3 hours to convert it to meta-kaolinite. 160 g of the meta-kaolinite was activated using a mixture of NaOH and commercial grade of Na_2SiO_3 in a ratio 1:1. For pure geopolymer, the percentage of NaOH was varied at 4%, 8%, 12% and 16% respectively. Pure geopolymer activated with 12% NaOH was observed with the highest value of compression strength and this percentage of NaOH was adopted for subsequent composite fabrication. 1.6 g of the 425 mm mesh size of cornstalk fibre was thoroughly mixed with 158.4g of meta-kaolin (1% fibre of 160g composites). The mixture was activated using mixture of 12% NaOH and commercial-grade Na_2SiO_3. The mixture was transferred into a wooden mould of 50 mm x 50 mm x 50 mm and left for 48 hours. The moulded bricks were made to pass through ambient curing for 10 days with 24 hours interval check of loss in weight. Compression strength of the brick was determined. The experiment was repeated for 2.5%, 5.0%, 7.5% and 10% of cornstalk fibre for the same size and also for 1.00 mm fibre size respectively[9,12]. The general experimental procedure is illustrated in Figure 1.

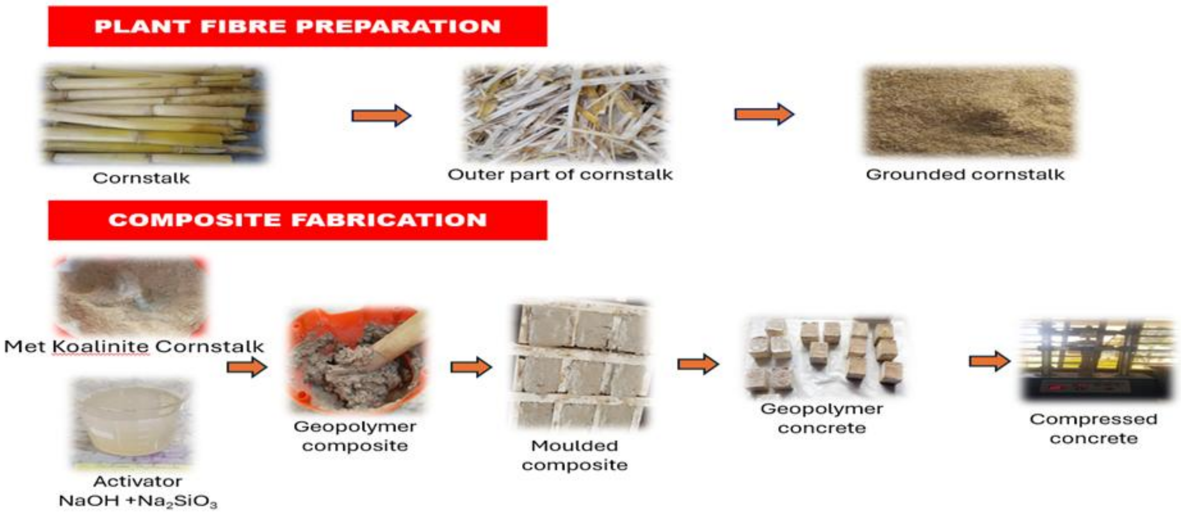

Figure 1: Sample Preparation and Treatment of Cornstalk Fibre

3. RESULTS AND DISCUSSION

Physical properties such as density, moisture content, hot and cold solubility, lignin content, cellulose content, 1% NaOH solubility were analysed to compare the corn stalk fibre with other lignocellulose fibre. Finally, the effect of the reinforcement was observed by carrying out the compressive strength of the composite concrete fabricated.

Table 1: Mean values obtained for Physical Properties of *Zea mays* stalk fibre

Lignocellulose fibre	Moisture contents (%)	Cold-water solubility (%)	Hot-water solubility (%)	1 % NaOH solubility (%)	Alcohol-benzene solubility (%)	Density (g/cm^3)
Zea mays stalk	10.00±0.50	20.50±2.18	25.50±2.50	45.30±2.52	11.50±1.73	0.39±0.03

Table 1 above, present the mean values obtained for physical properties of *Zea mays* fibre. The result obtained were in accordance with the result reported by Duraisamy *et al*., 2020[10]. According to literature, good lignocellulose fibre is expected to have low moisture content and low extraneous particles. The incorporation of fibre with considerably low extraneous particle in cementitious material composites as a reinforcement can enhance the flexural limit during splitting, durability, ductility, and break resistance compared to the unreinforced matrix[11].

Table 2: Mean values obtained for Chemical Properties of *Zea mays* stalk fibre

Lignocellulose fibre	Cellulose contents (%)	Lignin contents (%)
Zea mays stalk	49.67±0.58	35.30±3.51

Table 2 above, presents the mean values obtained for the chemical properties of *Zea mays* fibre. The results obtained were in accordance with the results reported by Okanlawon et al., 2022[9]. According to literature, good lignocellulose fibres are expected to have high cellulose content and low lignin content. The higher the cellulose content the better the plant for composite reinforcement[9].

Table 3: Mean values obtained for Compression test of Pure Geopolymer

Percentage of NaOH (%)	Volume ratio of NaOH to Na_2SiO_3	Ratio of Meta-kaolinite to Activator	Compression strength (Nmm^{-2})
4	1:1	2:1	9.07±3.80
8	1:1	2:1	8.93±3.40
12	1:1	2:1	10.93±1.89
16	1:1	2:1	3.20±2.78

Table 3 present the mean values obtained for compression strength of pure geopolymer. The result for compression strength of the pure geopolymer ranges from 3.20 Nmm^{-2} to 10.93 Nmm^{-2}. The pure geopolymer activated using 12 % NaOH was observed with the highest compression strength and this percentage of NaOH was adopted for subsequent geopolymer composites.

3.1. FTIR Characterization of cornstalk fibre

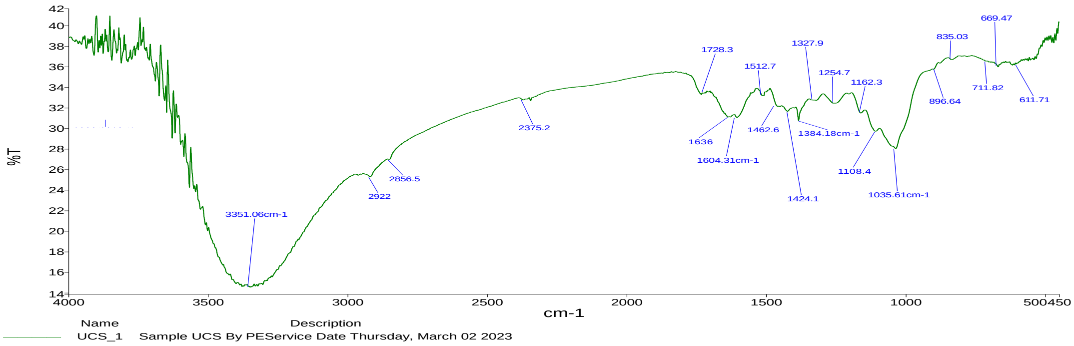

Figure 2: FTIR spectrum of cornstalk fibre

The FTIR spectrum of cornstalk fibre is shown in Figure 2. According to the spectrum, there is broad and strong band at 3351.06 cm^{-1} which could be attributed to –OH stretch of alcohol. Also, from the spectrum, there are sharp and narrow bands at 2922 cm^{-1} and 1728.3 cm^{-1}, assigned to -CH_2 pyranose structure and C=O carbonyl of cellulose respectively. Another prominent band in the functional group region were observed at 1636 cm^{-1} and 1604.31 cm^{-1}, which could also be attributed to H-O-H group. These bands observed in this spectrum confirmed the cellulose structure in cornstalk fibre which is common to all lignocellulose fibres and is in accordance with the result reported by[10].

Table 4: Mean values obtained for Compressive strength of geopolymer composite

Fibre size (mm)	Fibre volume (%)	Ratio of Fibre to geopolymer	Compression strength (Nmm^{-2})
0.425	1 %	1:150	15.52±0.92
0.425	2.5 %	1:60	19.46±0.95
0.425	5.0 %	1:30	22.12±1.13
0.425	7.5 %	1:20	26.65±1.05
0.425	10 %	1:15	38.80±1.19
1.00	1 %	1:150	18.02±0.72
1.00	2.5 %	1:60	20.91±1.03
1.00	5.0 %	1:30	25.53±0.74
1.00	7.5 %	1:20	29.42±2.03
1.00	10 %	1:15	41.74±0.48

Table 4 shows the results of the mechanical properties obtained from geopolymer composite reinforced with cornstalk fibre of different size and volume. The result indicates that the compression strength of the composite ranges from 15.52 Nmm^{-2} to 41.74 Nmm^{-2}. These results were in agreement with the result reported by[11]. According to the results the higher the ratio of fibre to geopolymer, the higher the compression strength of the geopolymer. However, the higher mechanical strength is observed with geopolymer reinforced with 1.00 mm fibre size compare to geopolymer reinforced with fibre size of 0.425 mm[4]. Figures 3 and 4 shows the curing time of reinforced geopolymer with different NaOH percentage, reinforced geopolymer with 0.425 mesh size and 1nm mesh size cornstalk fibre respectively. The figures shows that it took 7days for reinforced geopolymer with cornstalk fibre to cure, which indicates that as the days increases the weight of the bricks reduce drastically until it reach the point where the reduction difference is very low.

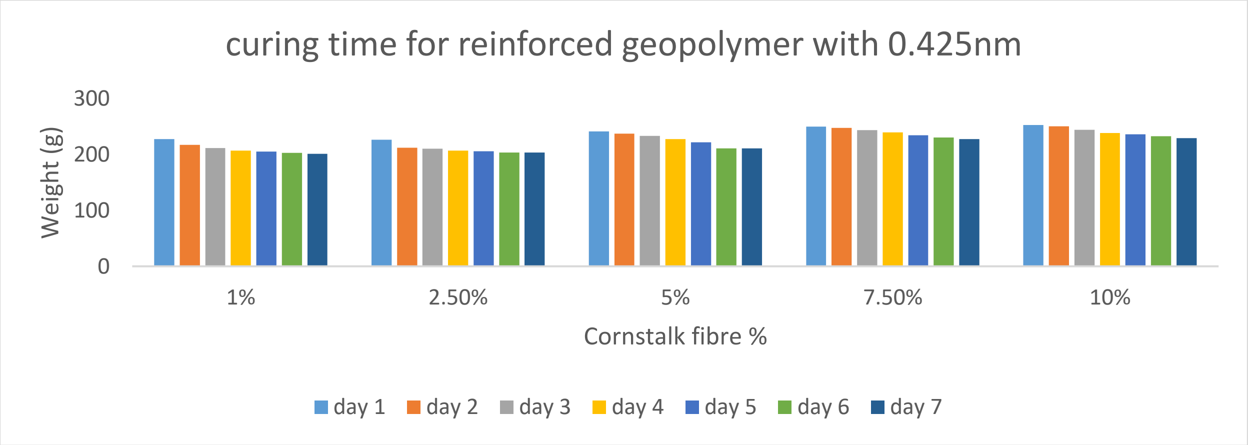

Figure 3: Curing time for reinforced geopolymer with 0.425nm mesh size cornstalk fibre

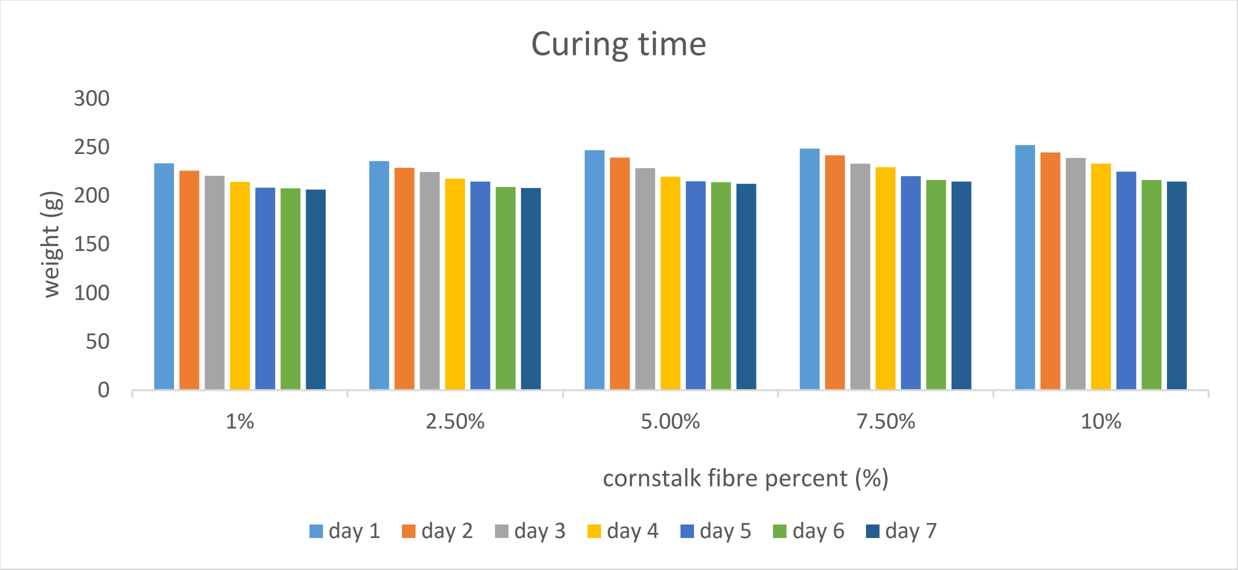

Figure 4: Curing time for reinforced geopolymer with 1nm mesh size of cornstalk fibre:

4. CONCLUSION

Corn stalk from agricultural waste can be utilized to create cornstalk fibre. The compressive strength of polymer composites with lignocellulose fibre are significantly affected by the properties of their components, the quality of bonding between the geopolymer and the lignocellulosic material. The characterization and compressive strength of corn stalk fibres revealed that they may be used as reinforcement for friction composites, which is an environmentally beneficial method of utilizing waste maize stalks. The usage of natural fibre is ought to be made more common. A code of practice should be created after looking at all the related issues to the reinforcement of natural fibre.

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Method Optimization for the Removal of Tetracycline from Water Using Bio-Waste Derived Nanomaterials

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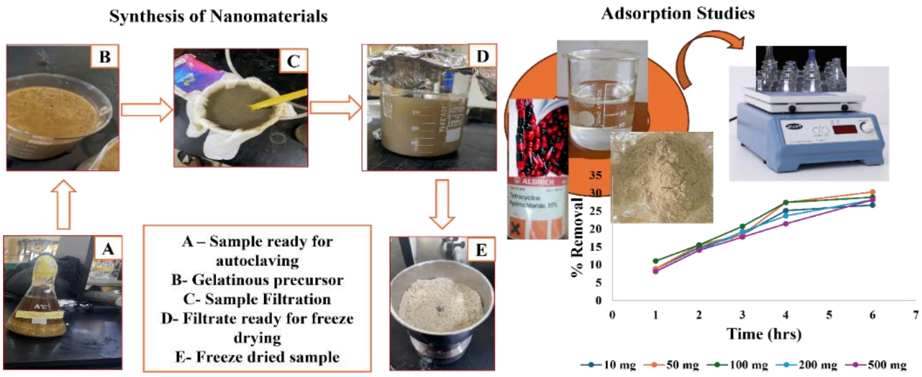
ABSTRACT

Pharmaceutically active compounds (PhACs) are essential chemical substances utilized for health maintenance and disease prevention. As a result of the widespread release, unregulated discharge and environmental effects of PhACs, there is a compelling need for efficient and sustainable removal strategies. This study focuses on the optimization of Tetracycline (TCN) removal from water using nanomaterials synthesized from potato peels. The adsorptive material used was prepared through chemical-hydrothermal processes and characterized using ATR-FTIR (Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy) and SEM (Scanning Electron Microscopy). FTIR analysis showed prominent peaks at 3272, 1643 and 1416 cm^{-1} which were associated with O-H, C=C isolated and C=C conjugated vibrations respectively in the nanomaterial while SEM analysis showed an irregular amorphous flat sheet with porous structures. Three wavelengths at 270 nm, 357 nm and 370 nm were tested in optimization studies for determination of TCN in standard solutions using UV-Visible spectrophotometer. Wavelength at 357 nm was observed as the optimal wavelength, which gave a linear calibration curve with a regression coefficient of 0.99 and absorbance values that ranged from 0.071 ± 0.001 to 0.185 ± 0.001 for the studied concentrations of 0.5 to 50 mg/L. There was no significant difference in the level of TCN adsorbed by the different doses of nanomaterials (10, 50, 100, 200, and 500 mg). However, removal efficiency was observed to be significantly time dependent. Overall, results indicate the potential of these biowaste-derived nanomaterials for tetracycline removal, offering a sustainable, eco-friendly, and cost-effective solution for water purification.



KEYWORDS: Antibiotics; Adsorption studies; Nanomaterial; Potato peel; Water purification.

1. INTRODUCTION

The increase in human pharmaceutical intake results in the daily release of pharmaceutically active compounds (PhACs) into the environment [1]. Since conventional wastewater treatment methods are unable to entirely remove pharmaceuticals from contaminated water, the discharge of wasterwater remains the main source of these organic micro-pollutants [2,3]. The presence of pharmaceuticals and the dangers associated with their metabolites or conjugates in the environment are issues that have become a growing source of global concern, even though PhACs normally occur at trace amounts in a range of environmental matrices [4].

In Nigeria where portable water is sourced from underground systems, there is the likelihood that these untreated wastewater will percolate into the soil and contaminate underground water [5]. The presence of PhACs in portable water have been linked to carcinogenicity and genotoxicity [6,7]. Furthermore, a number of PhACs, including hormones, anxiolytics, antibiotics, psychoactive compounds, and antiepileptic medications, are recognized to present serious threats to aquatic lives [8–10].

Antibiotics are being used extensively [11,12], which is causing their presence in a wider range of matrices and their environmental fate to become a global issue [13]. Multiple mechanisms such as electrostatic interaction, hydrophobic contact, hydrogen bonding, π-π interactions, and cation exchange, are involved in the adsorption of antibiotics from polluted water [14]. Carbon nanomaterials are an important class of adsorbent that excel in the electrostatic adsorption of antibiotics [15]. Previous studies have performed adsorptive removal of pollutants using chemically synthesized nanomaterials [16,17] while some studies synthesized nanomaterials for other purposes [18–20]. Here, we evaluated the adsorptive properties of nanomaterials derived from sweet potato peels for the removal of tetracycline (TCN) in spiked water by synthesising benign nanomaterials from potato peels, performing functional group and morphological characterization of the synthesized materials and testing the efficacy of the synthesised materials.

2. MATERIALS AND METHODS

2.1. Sample collection and synthesis

Sweet potato peels, used as carbon source, obtained from one of the kitchens within Chrisland University, were wrapped in aluminum foil, and kept in Zip lock® bags. The samples were washed several times with distilled water, then dried in a thermostated oven at 100 ℃ till constant weight, to ensure total elimination of moisture. After 29 hours, the hard and brittle sample was pulverized. 50 g of sample was then weighed and immersed in 2 L, 2% NaOH for 24 hours. Soaked potato peels were washed with distilled water, until completely neutral, autoclaved for 2 hours at 120℃, homogenized for 2 hours and filtered using a Buchner filtration set up. The filtrate was kept at 4°C prior to freeze-drying performed on a LYOTRAP lyophilizer.

2.2. Characterization of Freeze-dried materials

ATR-FTIR (Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy) was used to identify the presence of functional groups. The freeze-dried samples were evenly combined with KBr powder at a ratio of 1:100, ground down, and then compressed under 10 tons to create pellets. The disc containing the prepared pellet was then put inside the FT-IR spectrometer (MY19322004 Agilent Technologies Cary 630) disc holder. 32 scans were performed on the samples to get a transmission spectrum at 4 cm^{-1} resolution and a scanning range of 4000 cm^{-1} - 400 cm^{-1}.

The surface morphology and elemental analysis of freeze-dried samples were determined using field emission scanning electron microscopy with energy dispersive X-ray (SEM-EDX) (FEI Inspect F50 American Nancorporation).

2.3. Optimization and instrumental analysis of TCN

Stock standard solution containing 100 mg/L tetracycline (TCN) was prepared from a tetracycline hydrochloride (95%) standard (Sigma Aldrich). Calibration standards of 0.5, 1, 5, 10, 25 and 50 mg/L were prepared from the stock standard. Three different wavelengths (270 nm, 357 nm, 370 nm) were used to test the optimal wavelength for analysis of TCN in the standard solutions using a Biobase 752N UV-VIS spectrometer.

2.4. Adsorption studies

Batch adsorption study was performed employing different adsorbent dosage of nanomaterial against the same concentration of TCN. Here, 10, 50, 100, 200, and 500 mg of nanomaterial were introduced into separate conical flasks containing the same concentration of TCN (100 mL, 50 mg/L TCN). The sample material was then placed on an orbital shaker (ZD-9556) operated at an angular velocity of 130 rpm. After the first, second, third, fourth, fifth and sixth hour of shaking, 5 mL of aqueous sample was taken and centrifuged at 3000 rpm for 5 minutes. After which the supernatant was carefully separated from the solid residue and absorbance measurements were taken on the UV-VIS spectrophotometer. Procedural blanks were also analyzed.

3. RESULTS AND DISCUSSION

3.1. Functional groups and morphology of synthesised nanomaterials

Synthesized nanomaterials were composed of irregular flakelike sheets with porous structures (Fig. 1A, B & C). The FTIR spectra of synthesized nanomaterial in Fig. 1D revealed prominent bands at 3272 cm^{-1}, indicating the presence of O-H. Other important peaks present are 1643 cm^{-1}, 1416 cm^{-1} and 1080 cm^{-1}, which were associated with C-O, C=C isolated and C=C conjugated vibrations respectively in the nanomaterial.

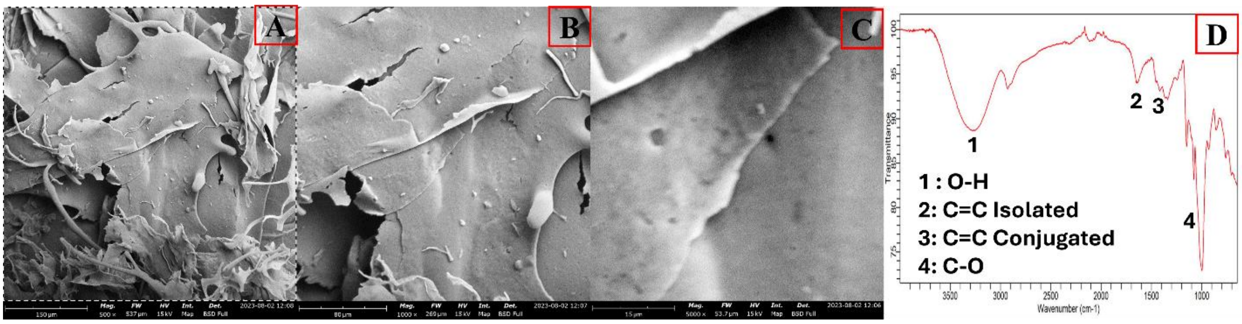


Fig. 1: Morphology of synthesized nanomaterials (A, B & C). FTIR wave bands showing prominent functional groups in synthesized nanomaterials (D)

3.2. Optimal wavelength for determination of TCN in spiked water

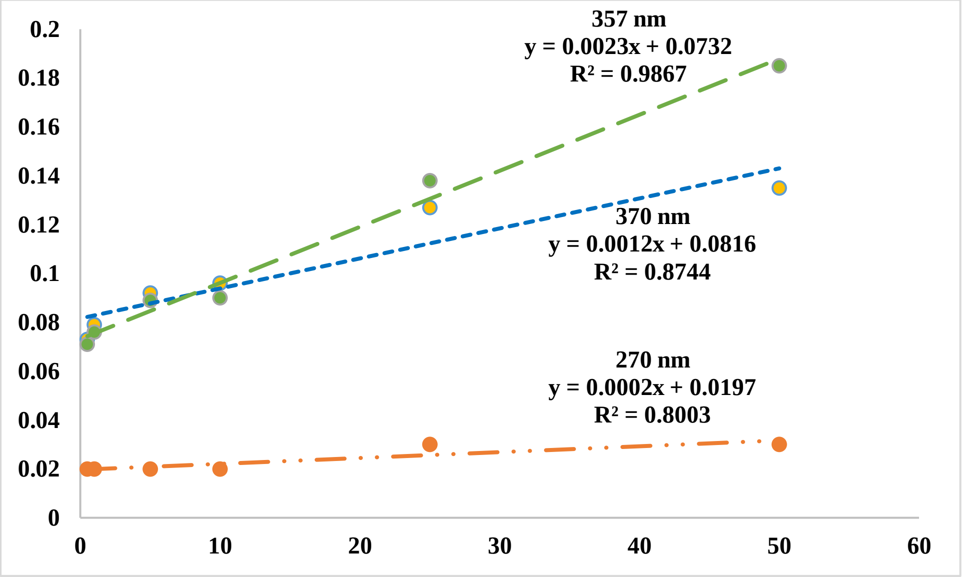


Fig. 2: Calibration plot at 270 nm, 357 nm, and 370 nm for TCN standard solutions

Triplicate analysis of tetracycline standard solutions gave linear calibration plot, for mean values, over the studied concentration (0.5 – 50 mg/L) range, with regression coefficients of 0.80, 0.87, and 0.99 at 270, 370, and 357 nm respectively. The wavelength at 357 nm was considered the optimal wavelength, having the highest r^2 value of 0.9867 (Fig. 2) with absorbance values that ranged from 0.071 ± 0.001 to 0.185 ± 0.001 for 0.5 to 50 mg/L TCN standard solutions.

3.3. Effect of adsorbent dose on the removal efficiency of TCN

Batch absorption studies revealed a continuous increase in the adsorption concentration of TCN in spiked water with increase in time for all quantities (10, 50, 100, 200, and 500 mg) of nanomaterials employed (Fig. 3). Although the adsorption efficiency was statistically indistinguishable among tested doses of nanomaterials ($p > 0.05$), 50 mg adsorptive material gave the best removal efficiency, having the highest increase at the sixth hour. Aside the first and third hour, where 100 mg adsorptive material gave slightly higher removal value than 50 mg, at every other time no adsorptive material had higher removal efficiency than 50 mg.

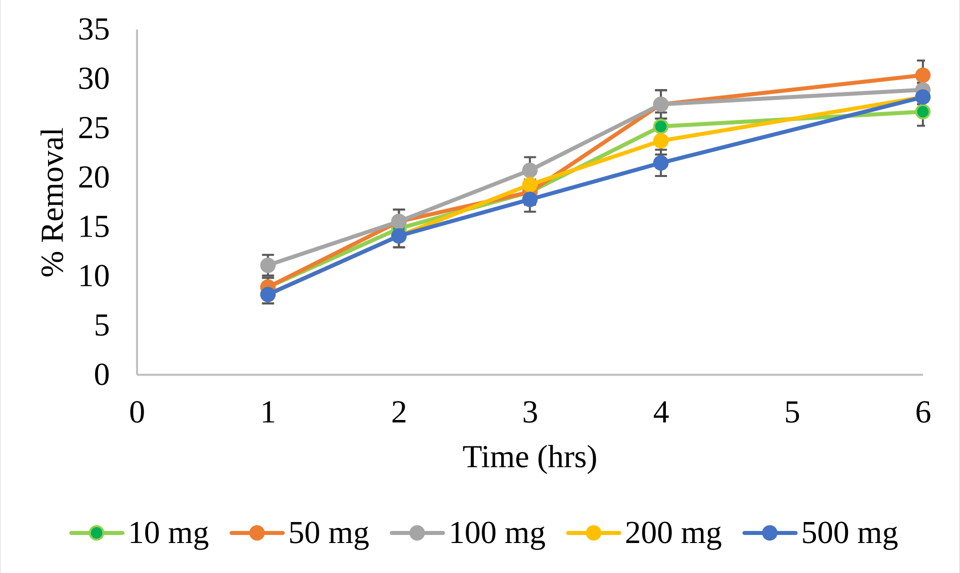


Fig 3: Percentage (%) removal of TCN from (50 mg/L) spiked water over six hours using different doses of nanomaterials. Error bars showing the mean values ± SD from triplicate determinations.

In addition, the other higher doses of adsorptive nanomaterials, viz., 200 mg and 500 mg, resulted in a decrease in removal efficiency across the studied time. The presence of O-H and C=C functional groups in our adsorptive nanomaterials is an indication of strong intermolecular attraction via hydrogen bonding and π-π interaction with TCN, which contains NH_2 functional group in addition to several OH and pi bond.

3. 4. Effects of adsorption time on the removal efficiency of TCN

Comparing the effect of time and adsorbent dosage on the adsorptive removal of TCN, it is obvious that that time had a more profound effect on the adsorptive removal of TCN from aqueous solutions than the adsorbent dosage. This suggests that the diffusion of tetracycline to the adsorptive site is crucial in the adsorption process [21]. In addition, the fact that percentage adsorption did not increase significantly with increase in adsorbent loadings shows that the mass transfer of TCN from the bulk solution to the surface of the adsorbent is slow and hence increasing the amount of adsorbent does not significantly increase the adsorptive removal of TCN [22] while adsorption time was found to significantly affect TCN removal efficiency. Our results revealed that TCN has a maximum adsorption capacity of 0.135 mg of TCN/mg.

4. CONCLUSION

This pilot study determined the efficiency of using nanomaterials synthesized from potato peels for the removal of tetracycline from spiked water. Overall, results indicate the potential of biowaste-derived nanomaterials for tetracycline removal, offering a sustainable, eco-friendly, and cost-effective solution for water purification. Further research employing more robust synthesis and characterization techniques as well as detailed removal studies using real wastewater samples, rather than spiked samples, are recommended.

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Synthesis and Tribological Evaluation of Polyol Based Biolubricant from *Spondias mombin (Anarcadiaceae) Linn*. Seed Oil

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ABSTRACT

Vegetable oil was extracted from *Spondias mombin* seeds and used to synthesize biolubricant via a two-step transesterification reaction. The biolubricant obtained showed an increased viscosity index (293.43), with improved pour point and cloud point properties (2°C and -9°C respectively). he biolubricant showed great potential in lowering the coefficient of friction (COF) (76.6 %) and its mean COF was observed to be within the acceptable range for automotive applications and comparable to commercially available engine oil (SAE20W40).

KEYWORDS: Tribology, *Spondias mombin*, FAME, biolubricant, trimethylolpropane.

1. INTRODUCTION

Lubricants consumed worldwide commonly originate from petroleum, coals, or natural gases[1]. Unfortunately, due to the world's enormous fuel consumption, these sources are limited and will eventually be depleted. This situation has raised interest among the scientific community in finding renewable green materials as replacements for the fossil[2]. With biofuels already gaining traction globally, attention has shifted to biolubricant products.

Biolubricants are chemically modified lubricating oils obtained from vegetable oils that have lubricating qualities comparable to mineral oil-based lubricants. As anti-friction agents, they are crucial for the optimal functioning of equipment by facilitating heat transfer, power transmission, lubrication, and corrosion inhibition[3]. Previous reports have shown that vegetable oil-based biolubricants provide better lubricity than petroleum-based oils[4]. The esterification of free fatty acids in the oil with methanol in the presence of acidic catalysts, and subsequent transesterification to biolubricants using trimethylolpropane (TMP) is the common chemical pretreatment for the conversion of vegetable oils to biolubricants[4,5]. The TMP has been widely reported for use in the formulation of biolubricants from vegetable oils due to the presence of branching which makes the biolubricants exhibit good thermal-oxidative stability and good low-temperature properties[6-8]. In this study, *Spondias mombin* seed oil was used to synthesize biolubricant via transesterification reaction with TMP and assessed for its tribological property.

2. MATERIALS AND METHODS

2.1. Materials and Solvents used

All reagents used were of analytical grade purchased from Sigma and Aldrich. *Spondias mombin* fruit seeds were obtained from Oro village in Isin L.G.A. of Kwara State, Nigeria. The sample was identified at the Herbarium, Department of Botany, Ahmadu Bello University Zaria, Nigeria. The flesh was removed from the seeds by washing with water and the kernels were sundried for 7 days, ground with a milling machine, sieved, and then stored in an oven at 40 °C to constant mass.

2.2. Extraction of seed oil

The pulverized seed sample (3 kg) was extracted by Soxhlet apparatus using n-hexane as the refluxing solvent at 69 °C based on the (NF V03-924) protocol. The crude oil was recovered under vacuum pressure using a rotary evaporator (Model No. HV-2199A, Hoverlabs/India). The extraction yields were evaluated gravimetrically thus;

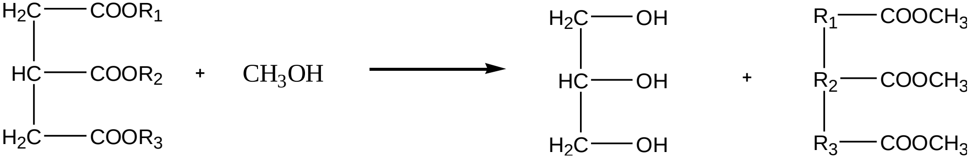
$$W = \frac{m_2 - m_1}{m_0} \times 100 \qquad (1)$$

where, W: extraction yields, m_2: mass of the balloon containing the extracted, m_1: mass of empty balloon, and m_0: mass of the vegetable oil sample.

The extracted oil coded SMSO was characterized for its physicochemical properties using standard methods[9]. The oil was also subjected to analyses using Gas chromatography-mass spectrometry (GC-MS) and Fourier transform- infrared spectroscopy (FT-IR).

2.3. Oil Esterification

The synthesis of fatty methyl esters (FAME) was carried out as reported by Ebtisam *et al*. [10]. The oil was initially esterified with methanol and H_2SO4 to reduce the free fatty acid and prevent saponification. After this, transesterification of the esterified oil was done to obtain fatty acid methyl esters (FAME).



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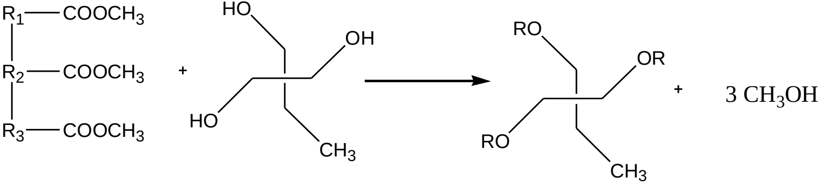
The synthesized FAME was characterised for pour point, cloud point, viscosity, and viscosity index, in addition to functional group analysis using Fourier transform infrared spectroscopy (FT-IR).

2.4. Synthesis of Biolubricant

Biolubricant was synthesized by transesterification of the methyl ester with TMP according to Ghazi *et al*.[11]. The synthesized biolubricant was characterized for physicochemical properties pour, cloud and viscosity, and viscosity index. It was also characterized for presence of functional group using FT-IR.



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2.5. Tribological Evaluation (Frictional Test)

The study of the coefficient of friction was carried out using a pin-on-disc tribometer in accordance with ASTM G-99 standards as reported by Thottackkad *et al*.[12]. A load of 10 N was applied at 10 cm/s and 5 min test duration and the temperature was maintained at 27 °C throughout the whole test process. The process was performed on the biolubricant, SAE 20W40 commercial lubricant and without lubrication.

3. RESULTS AND DISCUSSION

3.1. Physicochemical properties of vegetable oil

The results of the physicochemical properties of the seed oil are shown in Table 1.

The extracted *Spondias mombin* seed oil (SMSO) was observed to be strong and dark golden brown in colour with an oil yield of 10.9 % as shown is Table 1.

Table 1: Physicochemical properties of the extracted *Spondias mombin* seed oil (SMSO)

Seed Oil Property	Seed oil	FAME	Biolubricant
Colour	Dark golden brown	-	-
Oil Yield (%)	10.9	70.25	92.16
Density (g/cm^3)	0.826	-	-
Acid Value (mgKOH/g)	35.3	1.06	-
Free Fatty Acid (%)	17.6	0.53	-
Viscosity @ 40° C (*cSt*)	6.67	14.28	119.05
Viscosity @ 100° C (*cSt*)	1.90	3.81	30.48
Viscosity Index	47.95	167.48	293.43
Cloud Point (° C)	-	13	2
Pour Point (° C)	-	4	-9

Eromosele and Paschal[13] observed an oil yield as high as 31.5% using petroleum ether as the solvent of extraction. Moreover, Orhevba and Osho[14] obtained a closely related yield of *Spondias mombin* seed oil (10.8 - 13.4 %). This may be influenced by the moisture content of the seeds and temperature during extraction[14].

The percentage yield of SMSO biolubricant obtained was 92.2% as shown in Table 1 which shows that the transesterification process was close to completion. This was also similar to the yield (92 %) obtained by Sen *et al*.[7] and (94.12%) by Ifeanyi-Nze and Akhiehiero[15]. The specific gravity and density of the oil were 0.827 and 0.826 respectively. This value was closely related to results (0.825 gcm^{-3}) obtained by Orhevba and Osho[14] and (0.867 gcm^{-3}) by Eromosele and Paschal[13]. The kinematic viscosities of SMSO at 40 °C and 100 °C are presented in Table 1 and were determined to be 6.67 *cSt* and 1.90 *cSt* respectively. The viscosity index was also determined to be 47.95. Jumat[16] reported a viscosity of 74 for cotton seed oil and Shah *et al*. [17] reported a viscosity of 32 for *Jatropha curcas* seed oil also both at room temperature.

Improved kinematic viscosities were observed after transesterification of vegetable oil (6.67*cSt*, 1.90 *cSt*) to FAME (14.28 *cSt*, 3.81 *cSt*) at 40 °C and 100 °C respectively. Furthermore, an impressive increase in kinematic viscosity was observed at formulation with TMP with the biolubricant, exhibiting viscosities of up to 119.05 at 40 °C and 30.48 at 100 °C. This implies that the addition of TMP to the synthesized FAME improved the viscosity of the formulated biolubricant. The biolubricant can thus be classified as an ISO VG 100 lubricating oil according to the ISO standard 3448/ASTM D 2422 (ASTM, 2021). This also implies it is in the same class as SAE 30 oil, which is used for marine applications in a ship's engine room[18]. The VI of the SMSO biolubricant was determined to be 293.43.

The pour point and cloud point of the SMSO biolubricant were determined to be -9 °C and 2 °C respectively as shown in Table 1. It has been reported that the presence of ternary alcohols, such as TMP, diminishes the pour point and cloud point of biolubricant[19]. It is assumed that the presence of a large branching group at the mid-point of a fatty acid chain creates a steric barrier around the individual molecule and inhibits crystallization, the result of which is lower pour and cloud points [20].

3.2. GC-MS Profile of Vegetable oil

The GC-MS analysis was carried out on the vegetable oil to determine the fatty acid composition of the vegetable seed oil as shown in Table 2. The oil contained predominantly long-chain monosaturated fatty acids. Abiodun *et al*.[21] also reported the presence of Lauric acid, phenolic lipids and fatty alkanes in *Spondias mombin*.

Table 2: GCMS profile of extracted vegetable oil

RT	Fatty Acid	Area%	Profile
19.027	Lauric Acid	0.75	medium-chain saturated fatty acid
19.337	Oxirane, tetradecyl-	1.79	Fatty Acyls
20.882	Oleic Acid	59.4	long-chain monounsaturated fatty acid
21.067	9-Octadecenal	10.33	Lipids - Fatty Acyls
21.296	Carbamic acid ester	12.2	Carboxylic ester
22.888	Cresol	10.82	Phenolic lipids
31.461	2-(aminomethyl)-cis-cyclopentanol	0.74	Lipids - Fatty Acyls
31.548	11-Tetradecyn-1-ol acetate	0.88	Carboxylic ester
31.64	9-Octadecenoic acid (Z)-2-hydroxy-1-(hydroxymethyl) ethyl ester	0.32	Methyl esters
31.72	Cyclooctaneacetic acid, 2-oxo-	1.25	Carboxylic acids
31.8	Behenic acid	1.51	Long-chain saturated fatty Acid
		99.99	

3.3. FT-IR profile of Vegetable oil, Biodiesel and Biolubricant

As presented in Figure 1, absorption bands in the region of 2855–3000 cm^{-1} and 1375.4–1461 cm^{-1} are due to C–H stretching vibration, which indicates the functional group of alkanes in its molecular structural.

The vegetable oil was observed to have adsorption bands at 1744 cm^{-1} and 1710 cm^{-1} which indicate C-O stretching vibrations characteristic of carboxylic acids, which were observed to slightly shift to 1750 cm^{-1} after transesterification. This result gives evidence to the formation of the methyl ester as suggested by Ebtisam *et al*.[10]. The peaks at 1450 cm^{-1} and 1236 cm^{-1} are characteristic of the C=C and C-H stretching respectively

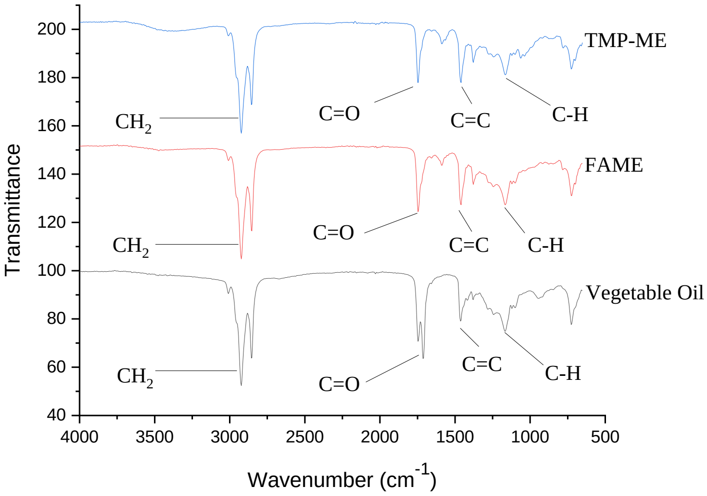

Figure 1. FTIR profile of vegetable oil (VO), Biodiesel (BD) and Biolubricant (TMP)

3.4. Tribological Evaluation

Generally, the biolubricant was observed to exhibit a coefficient of friction of 0.103 as shown in Figure 3. This value compares favorably with the value of conventional lubricant SAE20W/40 (0.106). The coefficient of friction of the biolubricant was to be within the acceptable range of coefficient of friction of a lubricant to be used for a wide range of automotive applications (0.01 – 0.14) reported by Habibullahi *et al*.[22]. As is common with biolubricants, the fatty acid constituent has an affinity for metallic surfaces in a tribo-pair which tends to make it reliable in reducing friction and wear. Also, the utilization of trimethylolpropane in the transesterification process results in removing the hydrogen molecules associated with beta carbon position which further results in improving the thermal stability of the TMP triester.

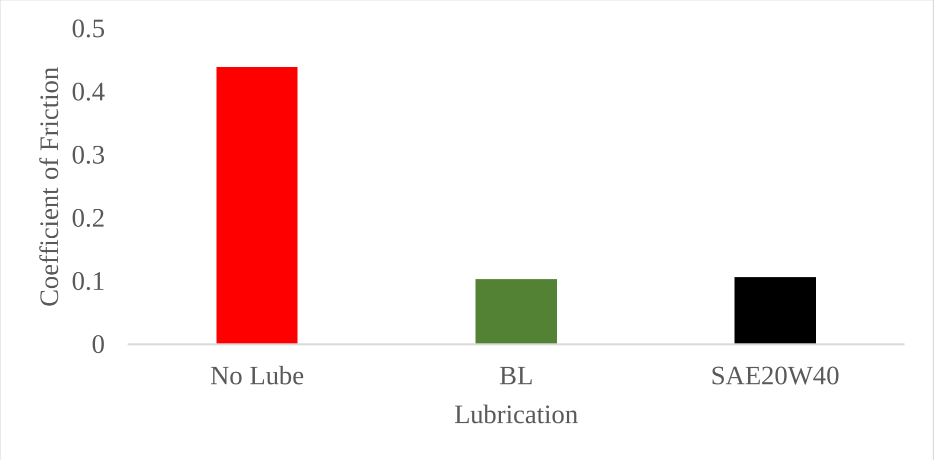

Figure 2. Mean coefficient of friction of biolubricant, standard lubricant and without lubrication

4. CONCLUSION

Vegetable oil derived from *Spondias mombin* seeds by soxhlet extraction was found to be rich in long-chain monounsaturated fatty acids, which makes it suitable for biolubricant production via transesterification with trimethylolpropane. The tribological evaluation of *Spondias mombin* based biolubricant demonstrated effective tribological solutions in moving parts of equipment, comparable to standard and commercially available lubricating oils.

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Synthesis and Performance Evaluation of Green Demulsifiers for Water-in-Crude Oil Emulsion Treatment

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ABSTRACT

The crude oil production industry faces a persistent challenge in the form of water-in-oil emulsions, necessitating the development of cost-effective and eco-friendly demulsification methods. This study presents a unique approach by synthesizing and characterizing green demulsifiers derived from coconut and soybean oils through a condensation reaction with diethanolamine catalyzed by p-toluene sulfonic acid. Characterization techniques, including Gas Chromatography-Mass Spectrometry (GC-MS), Fourier Transform Infrared Spectroscopy (FT-IR), and Thermogravimetric Analysis/Differential Thermal Analysis (TGA/DTA), were utilized. The demulsifiers' separation efficiency was evaluated through bottle tests with varying settling times, demulsifier dosage, and temperature. Results demonstrate the complete separation of stable water-in-oil emulsions by both Coconut Oil-Based Synthesized Demulsifier (COSD) and Soybean Oil-Based Synthesized Demulsifier (SOSD), albeit with differing kinetics. COSD exhibited superior separation kinetics, achieving 100% separation within 360 minutes at a 3 mL dosage, compared to 960 minutes required by SOSD under identical conditions. At 70°C with a 1 mL dosage, COSD achieved 100% separation in 30 minutes, while SOSD required 40 minutes. A comparative analysis with a chemical demulsifier (ethylene glycol) underscores the efficiency and viability of bio-based demulsifiers for emulsion breakage in the petroleum industry. These green demulsifiers offer environmental sustainability and cost-effectiveness. COSD demonstrates notably shorter separation times and lower required dosages, presenting a sustainable solution to the challenges posed by water-in-crude oil emulsions.

KEYWORDS: Green demulsifiers, Petroleum Flow Assurance, Emulsion treatment, Demuslsification, Coconut/Soyabean oil.

1. INTRODUCTION

The global crude oil industry continuously grapples with the challenge of water-in-crude oil emulsions, which reduce operational efficiency, increase costs, and pose environmental risks.[1–4.] During crude oil production, water is often produced alongside the oil, forming water-in-oil emulsions. These emulsions, consisting of water droplets dispersed throughout the crude oil, can cause several problems, such as increased viscosity, equipment corrosion, and flow obstruction in pipelines.[5] Traditional methods for demulsification rely heavily on synthetic chemical demulsifiers, which are often expensive, non-biodegradable, and toxic to the environment.[6–8]

The quest for greener alternatives has led to the development of demulsifiers from natural sources.[1,9,10] Green demulsifiers derived from plant extracts and vegetable oils are becoming increasingly popular due to their low environmental impact and biodegradability. This study focuses on the synthesis and characterization of two such green demulsifiers, derived from coconut oil (COSD) and soybean oil (SOSD). By leveraging the natural surfactant properties of these oils, this research aims to provide a sustainable solution to the water-in-oil emulsion problem in crude oil processing.

2. MATERIALS AND METHODS

2.1. Materials

Soybean seeds and coconut fruits were sourced from the National Institute for Pharmaceutical Research Development (NIPRD), Abuja, Nigeria. Crude oil was obtained from the Nigerian National Petroleum Cooperation (NNPC) in Port Harcourt, Nigeria. The reagents, p-toluene sulfonic acid, diethanolamine, petroleum ether, sodium chloride, and hexane, were of analytical grade, and procured from Finland Chemical Ltd, a subsidiary of Sigma-Aldrich.

2.2. Extraction of Soybean and Coconut Oils

Soybean oil was extracted using a Soxhlet extractor with hexane as the solvent. A total of 50 g of crushed soybean was placed in a thimble, and hexane was continuously cycled through the sample for 15 minutes. The extracted oil was recovered via rotary evaporation. For coconut oil, a hot extraction process was employed, where 1kg of blended coconut was mixed with 2.5L of water, filtered, and allowed to separate over 6 hours.[9,11] The separated coconut oil was then purified through heating and filtration.

2.3. Synthesis of Demulsifiers

Following the procedure previously reported,[12,13] the demulsifiers were synthesized using a condensation reaction of the extracted oils with diethanolamine, catalyzed by p-toluene sulfonic acid. The reaction was carried out at temperatures between 140°C and 180°C for COSD, and between 60°C and 150°C for SOSD. The reaction products were purified and stored for further analysis.

2.4. Characterization of Demulsifiers

All characterizations were performed at the Multi-User Science Research Laboratory of Ahmadu Bello University, Zaria, Nigeria. FT-IR and GC-MS were employed to characterize the molecular structure of the demulsifiers. Agilent FT-IR with Agilent MicroLab, transmittance against wavelength was analyzed at 8 resolution and 4000 – 650 range. The sample scan was done at 30 while the background scan was at 16. Similarly, Agilent GC-MS, 7890B model of GC and 5977 model MS were used with 99.999 percent Helium as carrier gas. Column: Agilent 19091S-433UI, HP-5ms Ultra Inert; 0°C—325 °C (350 °C): 30 m x 250 µm x 0.25 µm. Oven: the initial temperature of the oven was 40 °C with 2 min hold time, 1 min equilibration time and 325 °C maximum temperature. Thermal stability and degradation profiles were examined using TGA and DTA techniques. PerkinElmer thermal analyzer was used to analyze 19.876 mg 19.201 mg of COSD and SOSD demulsifiers respectively. The initial heating started at 30.00 ^{o}C to finial temperature at 750.00 ^{o}C at the rate of 10.00 ^{o}C/min for the demulsifiers.

2.5. Demulsification Bottle Tests

The demulsification process was carried out following the procedure by Saad et al.[3] and Yaakob and Sulaimon.[14] The performance of the synthesized demulsifiers (SOSD and COSD) was tested on a water-in-crude oil emulsion prepared with a 30:70 water-to-oil ratio. Bottle tests were conducted by adding varying amounts of demulsifiers (1 mL, 2 mL, 3 mL) to 10 mL of emulsion in 15 mL centrifuge tubes. The tubes were agitated and heated to 60°C in a water bath before allowing the emulsion to settle. The amount of separated water was recorded over time.

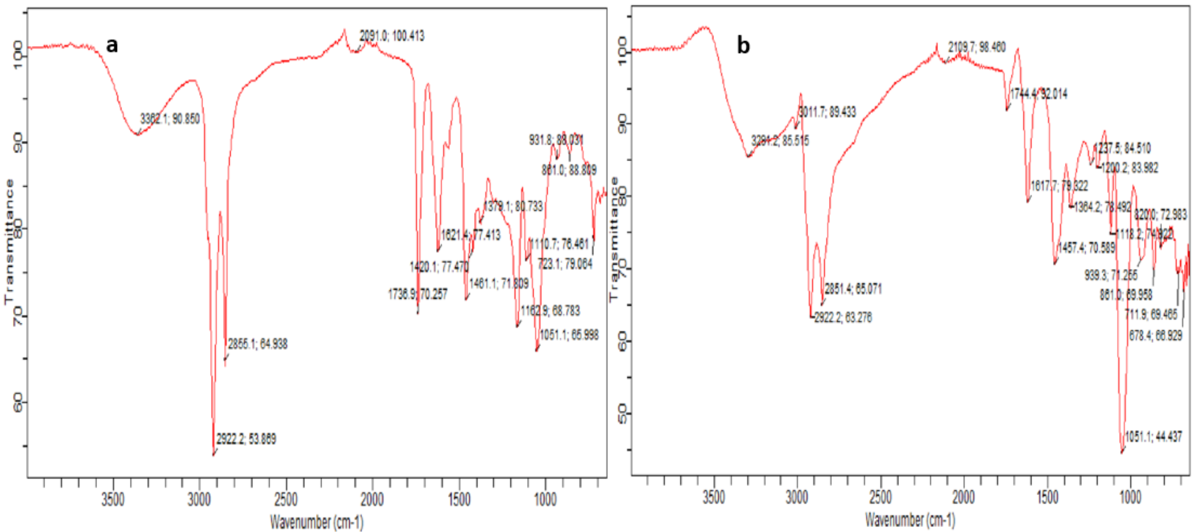
3. RESULTS AND DISCUSSION

3.1. Characterization Data of the Synthesized Demulsifiers

The characterization of the synthesized demulsifiers (COSD and SOSD) was conducted using several analytical techniques, including Fourier Transform Infrared Spectroscopy (FT-IR), Gas Chromatography-Mass Spectrometry (GC-MS), and Thermogravimetric Analysis/Differential Thermal Analysis (TGA/DTA). Each technique provided valuable insights into the chemical composition, functional groups, and thermal stability of the demulsifiers, confirming their suitability for applications in emulsion treatment.

3.1.1. Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR analysis was performed to identify the functional groups present in the synthesized demulsifiers. The spectra (Fig. 1) revealed characteristic peaks associated with hydroxyl (-OH), ester (C=O), and amine (-NH) functional groups, which are crucial for the surfactant properties of the demulsifiers. The presence of these functional groups indicates that the synthesis process effectively converted the coconut and soybean oils into functional demulsifiers. The peak intensities and positions can also give insights into the degree of substitution and the effectiveness of the condensation reaction with diethanolamine. FTIR spectra for COSD showed prominent peaks such as: 3362.1 cm^{-1}: O-H stretch of alcohols, 2922.2 cm^{-1} and 2855.1 cm^{-1}: C-H stretch of alkanes, 1736.9 cm^{-1}: C=O stretch of esters, and 1461.1 cm^{-1}: C=C stretch of aromatics. For SOSD, key peaks included: 3291.2 cm^{-1}: N-H stretch of amides, 2922.2 cm^{-1} and 2851.4 cm^{-1}: C-H stretch of alkanes, 1744.4 cm^{-1}: C=O stretch of ketones, 1200.2 cm^{-1} and 1237.5 cm^{-1}: =C-O-C stretch of ethers. Both results align with literature, confirming the presence of functional groups like esters, alcohols, and amides.[12,15]



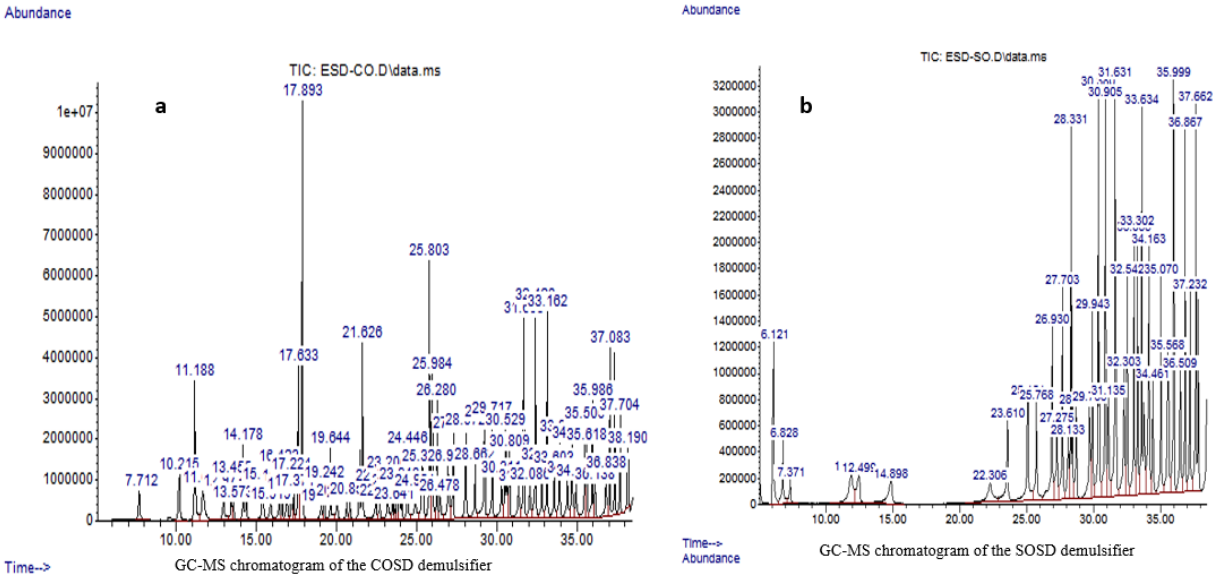
FT-IR Spectrum of the COSD Demulsifier

FT-IR Spectrum of the SOSD Demulsifier

Figure 1: FT-IR Spectra of the Synthesized Demulsifiers (a) COSD (b) SOSD.

3.1.2. Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis provided detailed information on the molecular composition and purity of the synthesized demulsifiers. The chromatograms (Fig.2) displayed a range of peaks corresponding to various components, confirming the successful synthesis of the desired products. Identifying specific compounds can be linked to the natural oils used and their transformation during the synthesis process. This analysis is critical for verifying the structural integrity of the demulsifiers and ensuring that no unwanted by-products were formed during synthesis. The GC-MS analysis of COSD identified 21 major components, including fatty acids, alcohols, esters, amides, ketones, hydrocarbons, and aldehydes. Notable compounds include octanoic acid (natural to coconut oil) and dodecanamide, N, N-bis(2-hydroxyethyl)-, a key amphiphilic demulsifier component. Similarly, SOSD revealed 15 major peaks, such as 9-eicosenoic acid and 2,3-dihydroxypropyl elaidate, indicating components derived from soybean oil and amidation reactions. Both demulsifiers exhibit hydrophilic and lipophilic regions, essential for effective demulsification. The result of the GC-MS was compared to results of the earlier research[3] and they showed some similarities in the composition. Saad *et al*.[1] reported esters, amines, hydrocarbons, carboxyles, amides, and fatty acids to be present in their corn oil based synthesized demulsifier.



GC-MS chromatogram of the COSD demulsifier

GC-MS chromatogram of the SOSD demulsifier

Figure 2: GC-MS Chromatograms of the Synthesized Demulsifiers (a) COSD (b) SOSD.

3.1.3. Thermogravimetric Analysis/Differential Thermal Analysis (TGA/DTA)

TGA and DTA were utilized to assess the thermal stability and decomposition behavior of the synthesized demulsifiers. The TGA thermograms (Fig. 3) indicated the weight loss of the samples as a function of temperature, allowing for the determination of thermal stability. COSD exhibited greater thermal stability compared to SOSD, with decomposition occurring at higher temperatures. This property is significant as it implies that COSD could perform effectively under the high-temperature conditions often encountered in oil production processes. The DTA results provided insights into the thermal transitions, such as melting and decomposition temperatures, which are essential for understanding the operational limits of these demulsifiers in practical applications. COSD remained thermally stable up to 64.48°C, with weight loss phases at 142.38°C (volatile components) and 346.64°C (fatty acid degradation), leaving 13.849% residue. SOSD exhibited similar behavior, stable up to 74.36°C, with decomposition phases at 149.64°C (moisture loss) and 380.33°C (organic degradation), leaving 9.023% residue. Both demulsifiers should avoid temperatures exceeding their degradation thresholds for optimal performance. TGA/DTA curves for both demulsifiers revealed two endothermic transitions: First peak - 91.45°C (COSD) and 104.83°C (SOSD), attributed to moisture vaporization; Second peak - 321.66°C (COSD) and 325.38°C (SOSD), associated with fatty acid decomposition. These transitions, confirmed by literature, emphasize the temperature-sensitive nature of the demulsifiers and their effective performance within stable thermal ranges.[16]

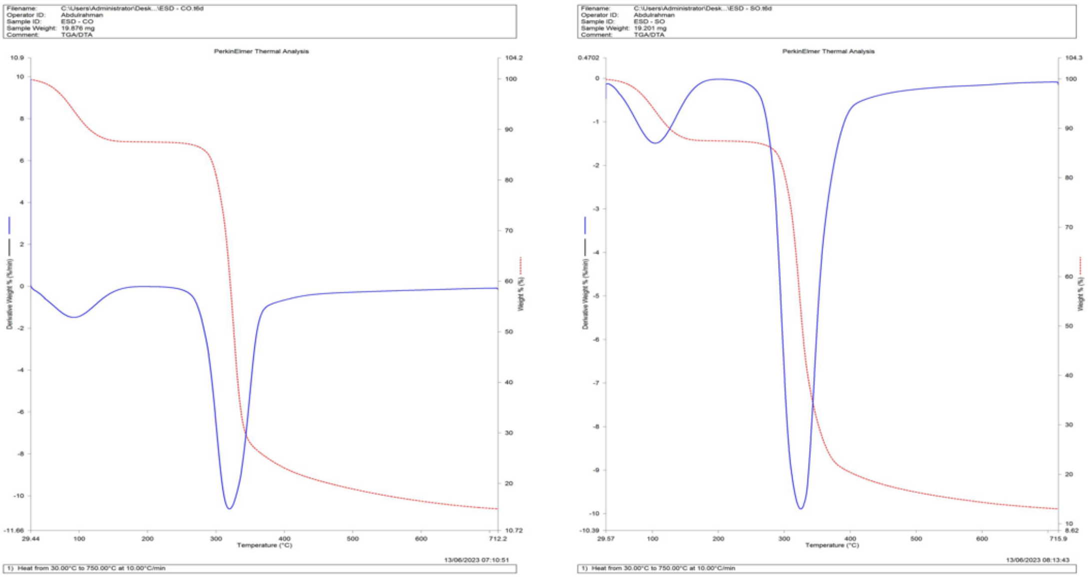


Figure 3: TGA/DTA Thermograms of the Synthesized Demulsifiers (a) COSD (b) SOSD.

3.2. Demulsification Studies

3.2.1. Effect of Dosage

The effect of demulsifier dosage was investigated using 1 mL, 2 mL, and 3 mL dosages. Higher dosages resulted in faster and more efficient separation. COSD achieved complete separation in 360 minutes with a 3 mL dosage, whereas SOSD took 960 minutes under the same conditions (Fig.4). This indicates that dosage plays a critical role in demulsification efficiency, with COSD requiring lower dosages for effective separation.

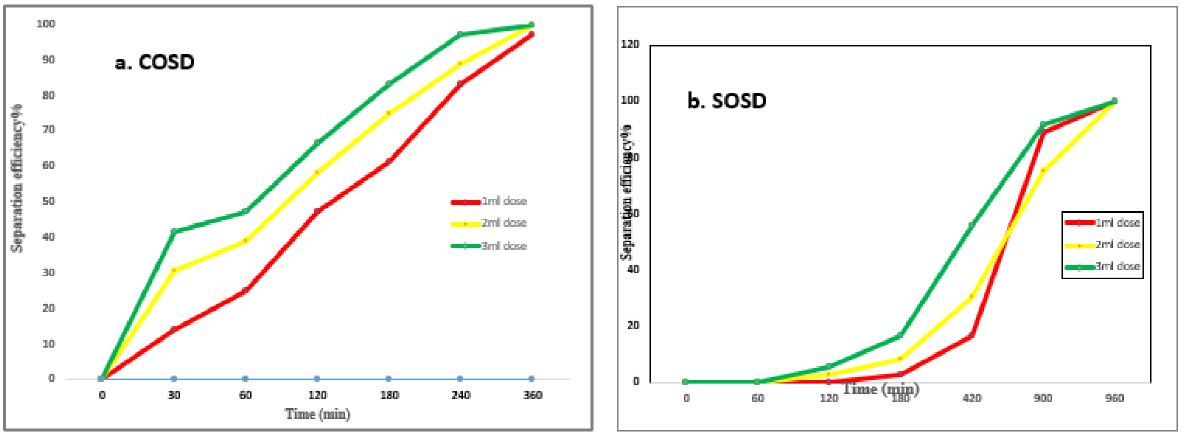


Figure 4: Effect of dosage on separation efficiency for COSD and SOSD demulsifiers

3.2.2. Effects of Settling Time

The settling time was another important factor influencing the separation efficiency of the emulsions. COSD outperformed SOSD in terms of separation speed, achieving complete separation within 360 minutes at the optimal dosage. The longer settling time required by SOSD (960 minutes) suggests that COSD is the more efficient demulsifier in terms of both time and performance (Fig.5).

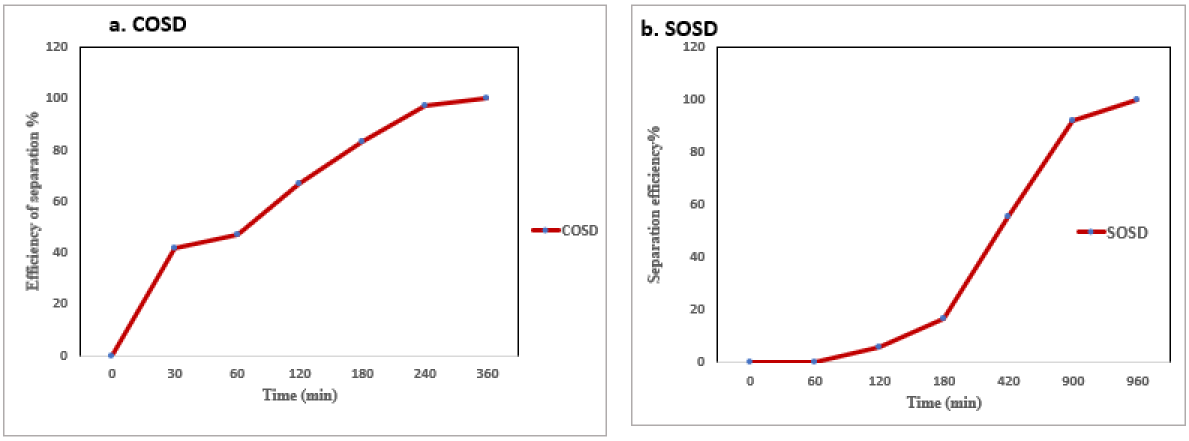


Figure 5: Effect of settling time on separation efficiency for COSD and SOSD demulsifiers

3.2.3. Effects of Temperature

Temperature significantly affected the separation process. At 70°C, COSD achieved 100% separation within 30 minutes at a 1 mL dosage, while SOSD required 40 minutes to reach the same level of separation (Fig. 6). The elevated temperature reduced the viscosity of the oil, allowing for quicker coalescence of water droplets and enhancing the efficiency of the demulsification process.

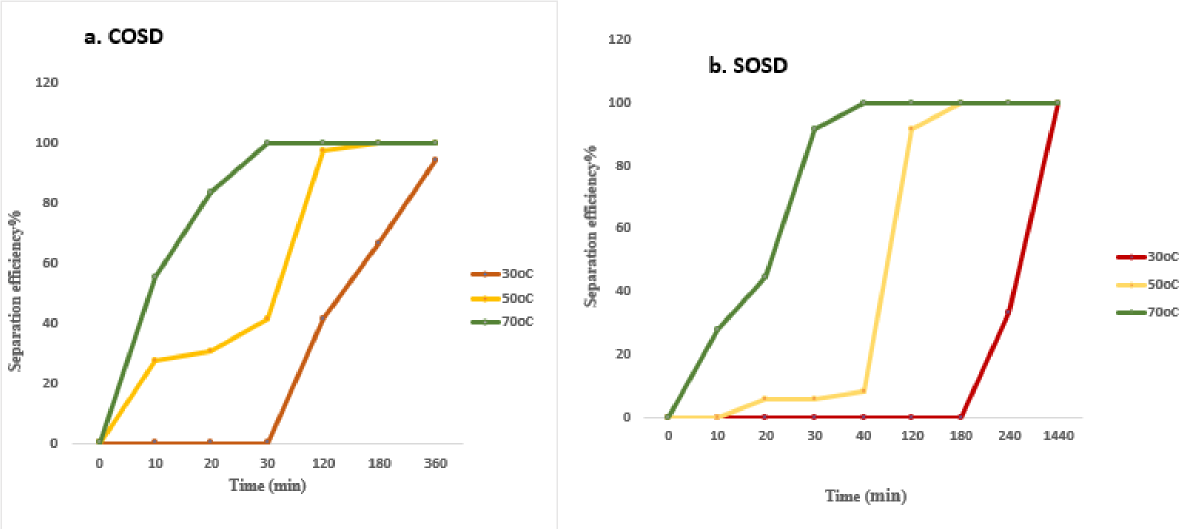


Figure 6 Effect of temperature on the demulsification efficiency of COSD and SOSD demulsifiers

3.3. Performance Evaluation of SOSD, COSD, and Chemical Demulsifiers

A comparative performance evaluation was conducted between COSD, SOSD, and a chemical demulsifier (ethylene glycol) (Fig. 7). COSD consistently outperformed both SOSD and ethylene glycol, particularly at lower dosages. COSD achieved complete separation at a 1 mL dosage and 70°C within 30 minutes, while ethylene glycol required longer times and higher dosages. This highlights the potential of COSD as an eco-friendly and efficient alternative to conventional chemical demulsifiers.

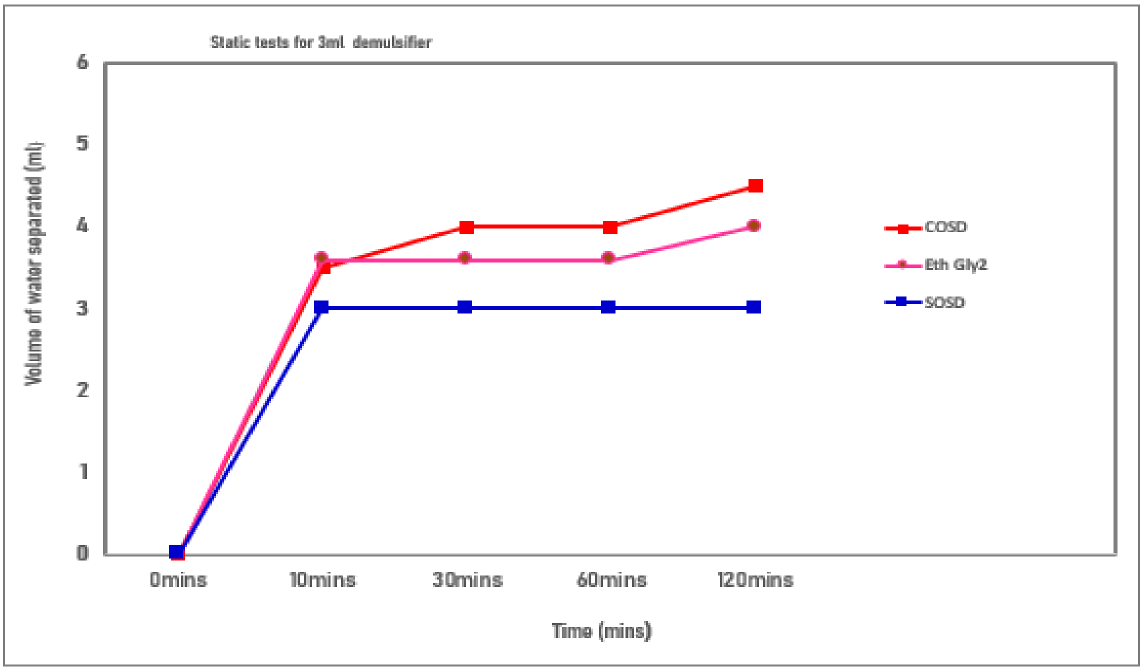


Figure 7: Performance evaluation studies of green and chemical emulsifier

4. CONCLUSION

The synthesis and characterization of COSD and SOSD as green demulsifiers have been successfully demonstrated. COSD, in particular, showed superior performance in terms of separation kinetics and dosage efficiency compared to SOSD and the chemical demulsifier (ethylene glycol). The use of renewable, non-toxic materials in the synthesis of these demulsifiers makes them environmentally friendly alternatives for the oil and gas industry. This study underscores the potential of bio-based demulsifiers in addressing the challenges associated with water-in-crude oil emulsions and supports the shift toward more sustainable industrial practices.

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Isolation and Characterization of Pyrrolizidine Alkaloids from *Heliotropium indicum* Leaf for Postharvest Management of Stored Grains

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ABSTRACT

The crude methanolic extract of *Heliotropium indicum* leaf was isolated and purified using chromatographic techniques by loading it on a glass column of length 100 cm and diameter 5 mm with 10 g silical gel (60-120 mesh) as the stationary phase. The compound caused approximately 98% mortality for *Sitophilus species* and *Callosobruchus maculatus* in a manner similar to standard insecticide (cypermethrin). Isolation was achieved in gradient elution system that started with 100% hexane followed by gradual increase of 5% ethyl acetate in hexane, then ethyl acetate, and lastly 5% methanol in ethyl acetate and were gradually increased by 5%.The separated fractions were examined using TLC. The pure IPM-65 isolate obtained as pure crystal was washed with methanol prior characterization using nuclear magnetic resonance spectroscopy, and the results were compared with standards in the literature. Liquid Chromatography Mass Spectrometry (LCMS) analysis revealed that the pure IPM-65 isolate was a mixture of pyrrolizidine alkaloids, including intermedine, supinine, lindelofine, and trachelanthine, as shown by their exact masses on the LCMS chromatogram.

KEYWORDS: Spectroscopy, Chromatography; Isolation; Characterization; *Heliotropium indicum.*

1. INTRODUCTION

Plants develop toxins as a type of secondary metabolite mainly for the purpose of self-defense against bacteria, fungi, insects and herb thieving enemies[1]. Well known bioactive compounds such as alkaloids, flavonoids, and terpenoids have major ecological functions and are responsible for preventing herbivores and microbial growth [2]. *Heliotropium indicum* L is a weed from the family Boraginaceae which is found almost everywhere and contains large amounts of secondary metabolites with considerable pharmacological value [3]. This plant grows as an annual or perennial herbaceous plant with a typical height of 15 to 50 cm and is widely distributed in the tropics and subtropics notably in Africa and Asia [4]. Distinct morphological traits like hairy stems and oppositely arranged leaves help this plant survive in a wide range of environments. In folk medicine, *H.indicum* is widely believed to serve many purposes including treatment for inflammation, wounds, and microbial infections because of its antimicrobial and anti-inflammatory properties[5].

Considering its bioactive component, this study aims to: (i) assess the effectiveness of pyrrolizidine compounds (IPM-65) obtained from leaf extracts of *H. indicum* on insect pests that affect stored grains, (ii) determine the structure of active compounds in the leaf isolate using nuclear magnetic resonance (NMR) spectroscopy and liquid chromatography mass spectrometry (LCMS), (iii) and measure the mortality rate of stored grains insect pests after application of pyrrolizidine compounds isolated from *H.indicum* leaf extracts. The pyrrolizidine alkaloid which is 65 mg of pure isolate obtained from methanolic leaf extract of *Heliotropium indicum* (IPM-65). It is a mixture of intermedine, supinine, lindelofine and Trachelanthine as confimed by their exact masses using liquid chromatpgraphy mass spectrometry technique. They are referred to as necine bases and are naturally occurring alkaloids based on the structure of pyrrolizidine. The necic acid moiety is joined to the necine base at position 9 (C_9) of the entire pyrrolizidine compound [6]. This study may help develop botanical insecticides as less harmful to the environment than chemical pesticides.

2. MATERIALS AND METHODS

2.1. Collection of Plant Sample

Fresh leaves of *H. indicum L* were collected from botanical garden of Benue State University, Makurdi, Benue State. The plant was authenticated at the herbarium section of the Forestry Research Institute of Nigeria (FRIN), Ibadan and a specimen copy with herbarium number **FHN/113768** was deposited [7].

2.2. Preparation of Crude Extract

The separated leaves were washed, air-dried for two weeks, and ground into coarse powder, then stored in an airtight bag. For extraction, 416 g of powder was soaked in 1248 mL hexane for 72 h, following [8]. The hexane extract (3.41 g) and subsequent ethyl acetate (2.17 g), methanol (9.98 g), and aqueous (7.72 g) extracts were obtained [7].

2.3. Isolation and Purification

A 9.0 g methanol leaf extract was adsorbed onto silica gel, dried, and packed into a glass column (100 cm x 5 mm) with 10 g silica gel in hexane. Gradient elution was used with increasing polarity starting with 100% hexane and 5% gradual addition of ethyl acetate in hexane, and methanol in ethyl acetate to yield 87 fractions. Fractions with similar Rf values were combined, washed with acetone, and TLC-tested (hexane: methanol 7:3, Rf 0.42). The compounds' identities were detected by spraying with Dragendorff reagents.Two alkaloid-positive fractions, IPM-85 (85 mg) and IPM-65 (65 mg), were obtained and prepared for NMR analysis [7].

2.4. Spectroscopic Analysis of Pure Isolate

The pure IPM-65 isolate was subjected to ^{1}H, ^{13}C, and 2D NMR (COSY, HSQC, HMBC) using a Bruker Avance III HD spectrometer with acetone as solvent. A 7 mg sample was dissolved in $CDCl_3$, filtered into a clean 5 mm NMR tube, and was analyzed at 400 MHz.TMS was set at 0 ppm which served as the internal reference, and spectra were phase- and baseline-corrected, yielding a complete NMR profile [7] .

2.5. Liquid Chromatography Mass Spectrometer (LCMS)

LCMS of pure IPM-65 was performed on an Agilent 6130 with 1200 series LC. A 1 mg sample in methanol was analyzed with a 1 mL/min flow rate, 3 mL injection volume, and 10-minute runtime. The ionization mode was ESI/APCI in positive/negative polarity, using an Agilent Poroshell 120 C_{18} column [7].

2.6. Insect culture protocols and Insecticidal activity screening of *H.indicum* extracts

Cleaned substrates were placed one-quarter full in kilner jars with added yeast and live adult insects, covered with muslin, and arranged on oil-filled trays to prevent predator access. After 14 days, adult insects were removed to assess emerging age [9]. Separately, 20 g of uninfected rice, sorghum, maize, and cowpea grains were treated with 0.01–0.08 g/mL of *H. indicum* hexane, ethyl acetate, and methanol extracts in jars. Ten pairs of young *S. oryzae*, *S. granarius*, *S. zeamais*, and *C. maculatus* were introduced, and insect mortality was assessed over four days using Abbott's formula [10].

$$\% \text{ Corrected Mortality} = \frac{T-C}{100-C} \text{x} 100 \qquad \text{Eqn (1)}$$

Where T= Treated mortality in %,C = Control mortality in % (Abbott, 1925)

$$\% \text{ Insect mortality} = \frac{\text{No of Dead Insects}}{\text{Total numbers of Insects for Infestation}} \text{ x } 100 \qquad \text{Eqn. (2)}$$

3. RESULTS AND DISCUSSION

3.1. Results

*3.1.1.*NMR Spectroscopy

The ^{1}H and ^{13}C NMR spectra of the pure IPM-65 isolate from methanolic extract of leaf of*H.indicum* L are given in (Figure 1) and (Figure 2) respectively, while important NMR parameters from the 2D-COSY NMR experiment on the isolate are given in (Table 1), which revealed the frequencies for a single isotope, most commonly hydrogen (^{1}H) along both x and y axes. The 2D COSY NMR parameters in Table 1 shows values indicating the chemical shift, and the coupling hydrogen [7].

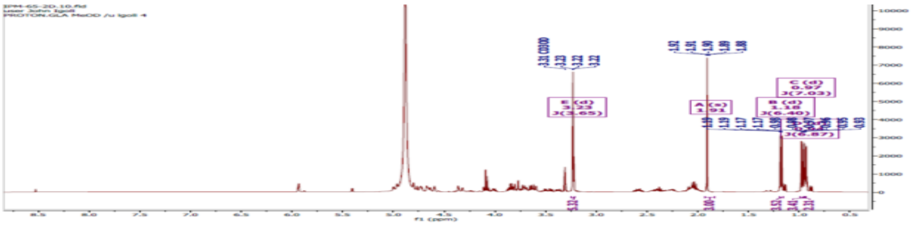

Figure 1: Proton NMR Spectrum of Pure IPM-65 isolate of methanolic extract of leaf of *H. indicum.*

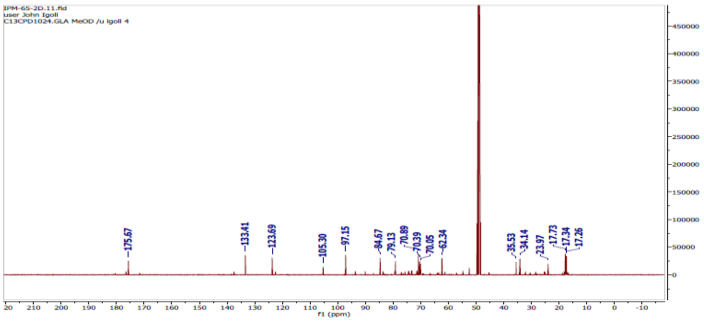

Figure 2:^{13}C-NMR spectrum of IPM-65solate of methanolic extract of leaf of *H. indicum.*

Table 1: NMR including 2D of pure IPM-65 isolate

Position	Atom	^{1}H NMR (ppm)	^{13}C NMR (ppm)	COSY/HSQC/HMBC
1	C=CH	5.90	123.69	2, 4.45, 4.65, 4.90
2	C=CH	5.45	133.14	3.45, 5.45, 5.90
3	CH$_2$	2.10	34.14	0.97, 2.55, 3.52, 3.58, 4.10
4	N	-	-	-
5	CH	3.45	70.05	5.45
6	CH$_2$	2.55	35.53	2.10, 3.80
7	CH	3.80	70.89	2.10, 2.55
8	CH	4.10	79.13	2.10, 3.23
9	CH$_2$	4.40	84.67	4.65
10	C=O	-	175.67	-
11	CH	3.23	62.34	3.60
12	CH$_3$	1.91 (s)	23.97	1.18, 1.91, 2.10
13	CH$_3$	0.97 (d)	17.34	0.97,2.10
14	CH$_3$	1.18 (d)	17.26	1.18, 4.10
15	CH	2.45	-	-
16	C	-	-	-

Based on the analysis of the proton and carbon spectra data and the 2D NMR compared with the reported data in literature, a new structure of pure IPM-65 isolate was proposed (Figure 3).

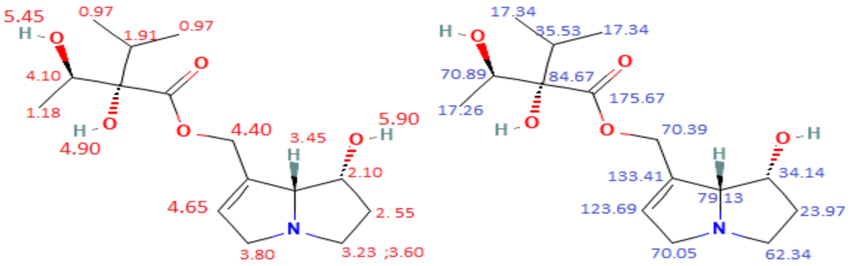

Figure 3: Structure of predicted Compound with assignment of proton and carbon

3.1.2. LCMS

Table 2 shows the result from LCMS chromatogram resulting from analysis of pure IPM-65 isolate run on High Resolution Electron Ionization Mass Spectrometry (HREI-MS) at University of Strathchlyde, Glasgow, Scotland, UK.In line with the proposed compound structure in (Figure 3), the proposed structure of pure IPM-65 isolate having pyrrolizidine skeleton were found to be that of Intermediine, Supinine, Lindelofine, and Trachelanthine based on their exact masses as revealed by LCMS.

Table 2: Exact masses of IPM-65

SN	Compd	E.M (g/mol)	M.F	RT	General name
1	Intermedine	299.1736 g/mol	$C_{15}H_{25}NO_5$	0.200	(7S, 8S)-7-hydroxy-5, 6, 7, 8-tetrahydro-3H-pyrrolizin-1-yl] methyl 2-hydroxy-2-[(1S)-1-hydroxyethyl]-3-methylbutanoate,
2	Supinine	283.1785 g/mol	$C_{15}H_{25}NO_4$	0.202	(8*S*)-5,6,7,8-tetrahydro-3*H*-pyrrolizin-1-yl]methyl (2*S*)-2-hydroxy-2-[(1*R*)-1-hydroxyethyl]-3-methylbutanoate.
3	Lindolefine	285.1923 g/mol	$C_{15}H_{27}NO_4$	0.205	2S,3R)-[(1R,7AR)-hexahydro-1H-pyrrolizin-1-yl]-methyl-2,3-dihydroxy-2-isopropylbutanoate,
4	Trachelanthine	301.1897 g/mol	$C_{15}H_{27}NO_5$	0.208	(1S,8S)-4-oxido-2,3,5,6,7,8-hexahydro-1H-pyrrolizin-4-ium-1-yl]methyl (2R)-2-hydroxy-2-(1-hydroxyethyl)-3-methylbutanoate

3.1.3. Insect Mortality

Table 3 shows percentage mortality of *Sitophilus* species and *Callosobruchus maculatus* exposed to pyrrolizidine compounds isolated from H.indicum leaf methanolic extract at 0.25 g/kg and 0.5 g/kg. At 0.5 g/kg, the pure isolate caused 98.33% mortality in cowpea weevils, 96.67% in maize weevils, 85% in sorghum weevils, and 81.67% in rice weevils within 96 h, comparable to cypermethrin ($P \leq 0.05$).

Table 3: Mortality of stored grain insect pests postexpoure to pure IPM-65 isolate

IPM-65	Conc. (g/kg)	substrate	Exposure time (Mean ±SEM)		
		Rice	Sorghum	Maize	Cowpea
@24 h	0.25	3.33±1.67^{a}	1.67±1.67^{a}	6.67±1.67^{a}	3.33±1.67^{a}
	0.5	6.67±1.67^{a}	8.33±1.67^{b}	10.00±0.00^{b}	6.67±1.67^{a}
@48 h	0.25	8.33±1.67^{a,b}	15.00±2.89^{c}	46.67±3.33c	51.67±6.00^{b}
	0.5	11.67±1.67^{b}	15.00±0.00^{c}	55.00±5.77c	58.33±9.27^{b}
@72 h	0.25	35.00±2.89^{c}	38.33±4.40^{d}	75.00±2.89^{d}	70.00±5.77^{c}
	0.5	46.67±1.67^{d}	48.33±1.67^{e}	81.67±1.67^{d}	81.67±1.67^{c}
@96 h	0.25	66.67±3.33^{e}	75.00±2.89^{f}	86.67±1.67^{e}	88.33±1.67^{d}
	0.5	81.67±1.67^{g}	85.00±0.00^{g}	96.67±3.33^{f}	98.33±1.67^{e}
Contr.	0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Cyp.	0.5 ml	68.33±4.41^{f}	76.67±6.67^{f}	96.67±3.33^{f}	100.00±0.00^{e}

The values are expressed as the mean ±SE at ($P \leq 0.05$) level of significance
Where Contr = Control, Cyp.= Cypermethri

3.2. Discussion

The characterization of IPM-65 as (1R,7aR)-1-hydroxy-2,3,5,7a-tetrahydro-1-H-pyrrolo[1,2-a]pyrrol-7-yl]methyl(2S,3R)-2,3-dihydroxy-2-isopropylbutanoate (intermedine) was confirmed through NMR and LCMS analyses. NMR spectra showed proton and carbon signals characteristic of a necic acid moiety and pyrrolizidine skeleton [11]. Key proton signals and COSY, HSQC, and HMBC correlations confirmed structural details, and LCMS identified the compound at 299.1736 g/mol [12]. Major PAs in *Heliotropium indicum* included supinidine, retrorsine, and echimidine, which are known for hepatotoxicity [13].

This study revealed the active potentials of this plant product as plant-derived insecticides against stored grain weevils, and provides a scientific rationale for the use of this botanical as alternative to synthetic insecticides in post harvest management of stored grains. The treatment with pure isolate of *H.indicum* had the highest percentage mortality of 98.33% for cowpea weevils. This was supported by the report of Adedire and Lajide [14] on *Piper guineense* belonging to the piperaceae family and stated that the plant possesses some forms of insecticidal properties against the eggs of stored cowpea grains, (bruchid) which are capable of suppressing various developmental instars of *Callosobruchus maculatus,* while Fasakin and Aberejo [15], have also reported that pulverized plant material from *P.guineense* inhibited egg hatchability and adult emergence of *Dernlestes maculatus* during storage.

4. CONCLUSION

The isolation and characterization of pyrrolizidine alkaloids as intermedine, Supinine, Lindelofine and Trachelanthine were successfully carried out from the leaf methanolic extract of *Heliotropium indicum.* The chemical identification of these compounds was validated using NMR and LCMS as pyrrollizidine alkaloids (PAs). It can be concluded that this plant has the potential as biopesticides against the insect pests of stored grains. Further research on the toxicity of this plant is therefore recommended to ensure its safe use as potential biopesticides in the postharvest management of stored grains.

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BOOK OF PROCEEDINGS
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Synthesis, Characterization, and Antibacterial Activity of Chitosan Crosslinked Ethylenediaminetetracetic Silver Nanocomposite (CCESN)

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ABSTRACT

The increasing resistance to antibiotics has necessitated the development of alternative antimicrobial agents. This study synthesized silver nanocomposites using chitosan synthesized from shells of *Archachatina marginata*. Chitosan was crosslinked with EDTA, serving as both a reducing and capping agent. The resultant product was characterized through Energy Dispersive X-ray (EDX), Transmission Electron Microscopy (TEM), X-ray Diffractometry (XRD), and Ultraviolet-Visible (UV-Vis) Spectrometry. The silver nanoparticles formation was initially shown by a colour change to brown which was further established by a peak absorbance at 435 nm, from a UV-Vis spectrophotometry scan. TEM and XRD analyses confirmed that the silver nanocomposite has a face-centred cubic structure, with an average size of 45.30 nm. EDX analysis identified silver as the primary element in the nanocomposite. The antimicrobial efficacy of the silver nanocomposites was tested against strains of *Escherichia coli* O157 and *Staphylococcus saprophyticus* DSM 18669. The antimicrobial tests revealed higher susceptibility of *Staphylococcus saprophyticus* DSM 18669 compared to *Escherichia coli* O157, demonstrating the nanocomposites' targeted efficacy. Future studies should investigate the molecular mechanisms of antibacterial activity while exploring scalable production and biocompatibility for applications in healthcare, food preservation, and wound care.

KEYWORDS: Nanocomposite, antimicrobial resistance, chitosan, nanoparticles, nanomaterials.

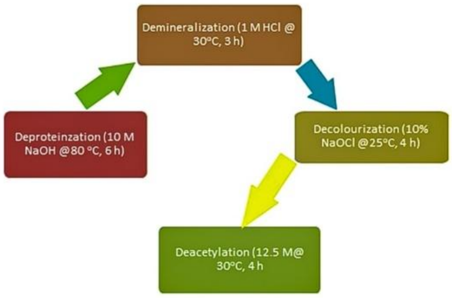
1. INTRODUCTION

Nanotechnology, which involves the manipulation of materials at the molecular scale, has found diverse uses in areas such as biomaterials, nanomedicine, and energy[1]. However, traditional methods for synthesizing nanoparticles often employ toxic chemicals[2], prompting an increased interest in eco-friendly biological approaches[3]. Among these, silver nanoparticles (AgNPs) are known to possess strong antimicrobial properties, though their precise mechanisms remain under investigation[4]. Chitosan, a biodegradable and biocompatible polymer derived from the shells of crustaceans and molluscs, is especially useful in nanoparticle synthesis[5]. In this work, chitosan from the shells of *Archachatina marginata*, combined with ethylenediaminetetracetic acid (EDTA), was used to produce silver nanoparticles. The primary objective of this study is to determine the characteristic properties of a synthesized chitosan-EDTA silver nanocomposite, and evaluate its antibacterial efficacy against *Staphylococcus saprophyticus* DSM 18669 and *Escherichia coli* O157, with the aim of developing sustainable nanomaterials and addressing antibiotic resistance.

2. MATERIALS AND METHODS

2.1. Chitosan Synthesis

Chitosan was obtained from *A. marginata*'s shell via deproteinization, demineralization, decolorization, and deacetylation processes[6], as outlined in Scheme 1.



Scheme 1: Synthesis of chitosan from the shells of *Archachatina marginata*

2.2. Preparation of Cross-Linked Chitosan-EDTA

Chitosan (1 g) was dissolved in acetic acid (100 mL), and EDTA (0.34 g) was added to form cross-linked chitosan-EDTA, which was then separated from the supernatant via centrifugation[7].

2.3. Synthesis of Chitosan-EDTA Silver Nanocomposites (CCESN)

The cross-linked chitosan-EDTA suspension (0.001%) was mixed with silver nitrate (5 mM), the pH was adjusted to 10 with NaOH, and the reaction proceeded for five hours to synthesize CCESN[7].

2.4. Characterization of CCESN

The synthesized CCESN was characterized using UV-Vis spectrophotometry, TEM, XRD, and EDX.

2.5. Antibacterial Study of CCESN

Antibacterial activity of the synthesized CCESN was assessed against *S. saprophyticus* DSM 18669 and *E. coli* O157 using disk diffusion and broth microdilution methods[8].

2.6. Statistical Analysis of Antibacterial Studies

The results of antibacterial studies were analyzed for statistical significance using ANOVA in GraphPad Software Inc., with triplicate measurements expressed as mean ± standard deviation.

3. RESULTS AND DISCUSSION

The study confirmed the successful formation of AgNPs, as evidenced by a visible color change (Figure 1) and a UV-Vis absorption peak at 435 nm (Figure 2). While the color shifts were less pronounced compared to earlier reports, they still indicated nanoparticle formation[9]. The distinct 435 nm peak, attributed to Surface Plasmon Resonance (SPR), further confirmed AgNP synthesis[10]. Additionally, peaks between 200–300 nm, corresponding to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions, suggested chelation between chitosan and EDTA[11]

Figure 1: CCESN solution after completion of the synthesis

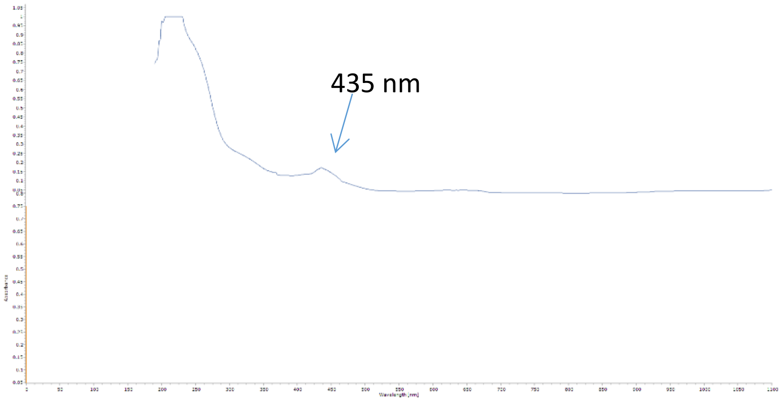


Figure 2: UV-Vis spectrum of synthesized CCESN highlighting the surface resonance plasmon effect

TEM images revealed spherical, polydisperse nanoparticles with an average size of 45.30 nm (Figure 3). The minimal particle aggregation suggests that the chitosan-EDTA cross-linking provided a stabilizing layer[12].

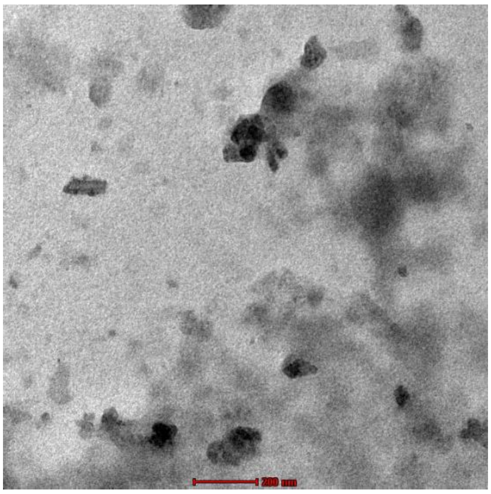


Figure 3: TEM micrograph of synthesized CCESN (200 nm)

XRD patterns (Figure 4) confirmed the presence of crystalline silver particles, with peaks at 38.62°, 42.95°, and 45.99° corresponding to the (111), (200), and (220) planes of the face-centered cubic lattice structure[13]. The crystallite size was calculated to be 22.20 nm[13]. Weaker peaks, likely due to the semi-crystalline nature and lower concentration of chitosan and EDTA, were also observed[14]

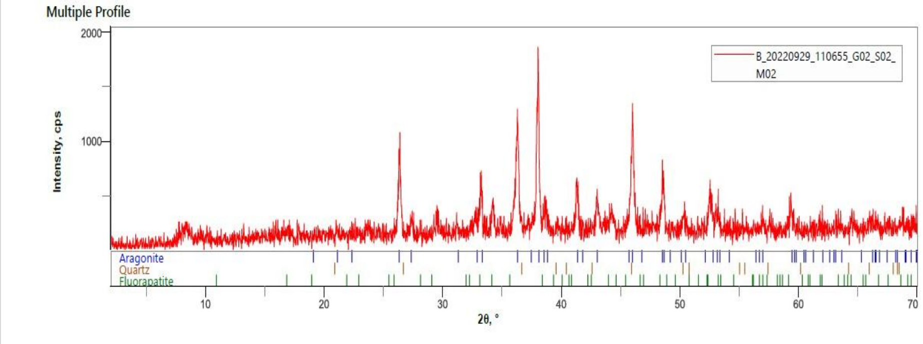


Figure 4: XRD pattern of synthesized CCESN

EDX profile (Figure 5) confirmed the presence of elemental silver (2.0–3.6 keV), aligning with UV-Vis and XRD results. Additional elements such as nitrogen, sulphur, copper, and iron were also detected, likely originating from chitosan, EDTA, or minor impurities[15,16].

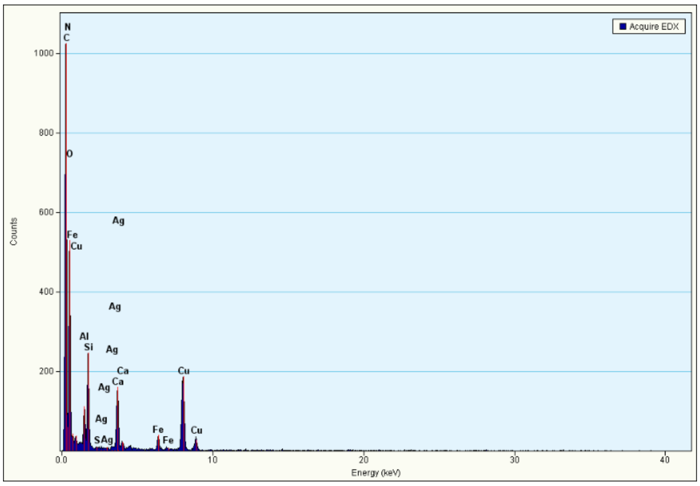
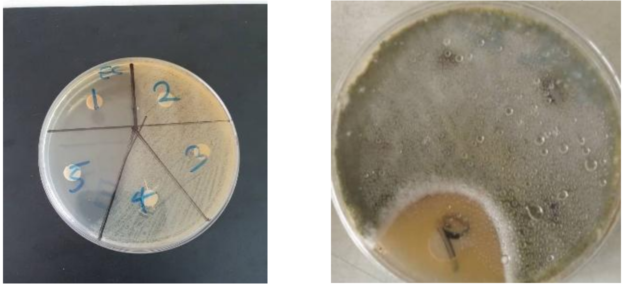


Figure 5: EDX profile of synthesized CCESN

The synthesized CCESN exhibited significant antibacterial efficacy, particularly against *S. saprophyticus* (46 mm), outperforming Levofloxacin at all tested concentrations[17]. (Figures 6a-b, Figures 7a-b;Tables 1-2). While CCESN was slightly less effective than Levofloxacin at the highest concentration, it demonstrated comparable efficacy at lower concentrations.



a b

Figure 6: Zone of inhibition of the synthesized CCESN against (a) *S. saprophyticus* DSM 18669 (b) *E. coli* strain 0157

c d

Figure 7: Zone of inhibition of Levofloxacin (c) *S. saprophyticus* DSM 18669 (d) *E. coli* strain 0157

Table 1: Inhibition zone for *S. saprophyticus* DSM 18669

Sample	Concentration (mg/mL)	Zone of inhibition (mm)
Silver nanocomposite	40	46.0^{a} ±0.82
Levofloxacin	0.05	31.33^{c}±0.4
	0.025	27.33^{c}±0.24
	0.0125	24.50^{c}±0.41
	0.0063	18.33^{b}±0.23

Columns with the different alphabets are statistically significant the same while those with same alphabet are statistically insignificant ($p<0.05$)

Table 2: Inhibition zone for *E. coli strain 0157*

Sample	Concentration (mg/mL)	Zone of inhibition (mm)
Silver nanocomposite	40	25.70^{a}±0.25
Levofloxacin	0.05	30.33^{b}±0.24
	0.025	25.50^{b}±0.41
	0.0125	20.17^{b}±0.24
	0.0063	0.00

Columns with the different alphabets are statistically significant the same while those with same alphabet are statistically insignificant ($p<0.05$)

The minimum inhibitory concentration (MIC) for both bacterial strains was 0.0781 mg/mL (Table 3), suggesting a bactericidal mode of action[17]. It is hypothesized that silver ions in CCESN interact with the negatively charged bacterial cell membrane, disrupting its integrity and inducing apoptosis[18,19].

Table 3: Minimum inhibitory concentration of the synthesized CCESN against the bacterial strains

Strains	MIC (mg/ mL)
Staphylococcus saprophyticus DSM 18669	0.0781
Escherichia coli strain 0157	0.0781

4. CONCLUSION

The chitosan-EDTA silver nanocomposite (CCESN) demonstrates potential as an effective and sustainable antibacterial agent, particularly against *S. saprophyticus*. While its efficacy against *E. coli* was slightly lower than Levofloxacin at the highest concentration, it performed comparably at lower doses. Future research should focus on optimization strategies and evaluate potential environmental and health risks associated with its application.

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BOOK OF PROCEEDINGS
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Green Synthesis of Silver Sulfide Nanoparticles from *Musa paradisiaca* Extract for Corrosion Inhibition of Mild Steel in 0.5 M Sulphuric Acid

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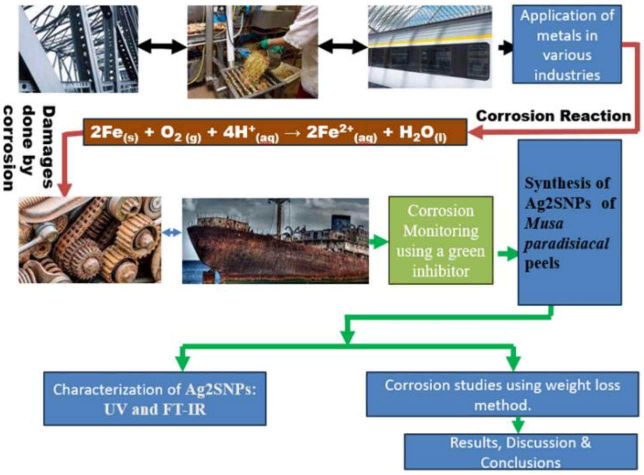
ABSTRACT

Corrosion attack affects the functionality of metallic materials in industrial installations. The material is usually protected from these effects by the use of inhibitors. As an alternative to synthetic chemical/inorganic inhibitors, researchers are exploring environmentally friendly solutions, such as natural products like essential oils, for protecting these metals against corrosion. Nanomaterials are increasingly being applied in various fields due to their effective properties. This study explores the green synthesis of silver sulfide nanoparticles using an aqueous extract of *Musa paradisiaca* peels as reducing, capping, and stabilizing agents for inhibition of corrosion of mild in sulphuric acid medium. The nanoparticles were characterized using Fourier Transform Infrared Spectroscopy (FTIR) and UV-Vis Spectroscopy. The UV-Vis spectrum revealed a strong emission peak at 400 nm, indicating the presence of Ag_2S nanoparticles. FTIR spectra showed functional groups such as alcohol/phenol –OH, carboxylic acid –OH, and N-H of amides, with a notable C=C stretch at 1620 cm-^{1}. The synthesized nanoparticles effectively reduced corrosion when applied to mild steel. The study found that higher nanoparticle concentrations and higher temperatures enhanced their protective effects, suggesting a strong chemical interaction between nanoparticles and the metal surface. This protective mechanism aligns with fundamental principles of chemical adsorption. Adsorption isotherms, including Tempkin Langmuir, Freundlich, and Adejo Ekwenchi, confirmed the chemisorptive nature of the process. Overall, this research highlights a promising pathway for developing eco-friendly corrosion inhibitors using natural resources and advanced nanotechnology.



KEYWORDS: Synthesis, Corrosion, Nanoparticles, *Musa paradisiaca*, Isotherms.

1. INTRODUCTION

Finding corrosion inhibitors that are environmentally safe and readily available has been a growing trend among scientists and engineers. Among the known nanoparticles, silver nanoparticles (Ag-NPs) are most commonly and widely used. [1-11] due to their versatility of applications. Studies have shown that sulfidation of Ag-NPs effectively reduces its toxicity. However, the ease with which Ag_2S-NPs may transform in some environments leading to the initial rapid release of dissolved Ag(I) and subsequent formation of Ag^0-NPs suggests that Ag_2S-NP is highly safe [12]. Several research has been carried out to evaluate the medicinal and nutritional potent, traditional anti-diabetic agent, and many other pharmacological activities of *Musa paradisiaca*. [13] The extract of this plant converted to silver sulfide nanoparticles, which contain many environmentally friendly compounds, may be utilized as eco-friendly corrosion inhibitors. This present study investigated the inhibitive potential of *Musa paradisiaca* peels silver sulfide nanoparticles through thermodynamic, kinetic, and adsorptive parameter studies.

2. MATERIALS AND METHODS

2.1. Plant extraction and green synthesis of silver sulfide (Ag_2SNP) using *Musa paradisiacal* peels

Musa paradisiaca peels, authenticated at the Department of Biological Sciences, Benue State University, Makurdi, were dried to a constant weight after 7 days. After crushing and pounding into powder, 20 g was weighed into an 800 mL conical flask, and 200 mL of distilled water was added. The mixture was boiled for 10 minutes using a heating mantle/hot plate. The solution, thereafter, was cooled and filtered into a 250mL conical flask to obtain an aqueous extract.

Green synthesis of silver sulfide nanoparticles was done by reacting silver nitrate solution with *Musa paradisiaca* peels aqueous extract and sodium sulfide at 27 ^oC using 1g of silver nitrate dissolved in 100 ml of *Musa paradisiacal* peels aqueous extract under magnetic stirring for 10 minutes. A solution of sodium sulfide containing 1 g was prepared in 100 ml of distilled water and gently swirled for homogeneity. The sodium sulfide solution was then added dropwise to the mixture of $AgNO_3$ solution and *Musa paradisiaca* peel extract under continuous magnetic stirring until the color of the solution changed to a suspended gray-black color, an indication of the formation of silver sulfide nanoparticles. [14] Thereafter, the mixture was centrifuged at 8000 rpm for 4 hours, the supernatant removed, and the precipitate collected and rinsed with 5 mL of distilled water. The obtained nanoparticles were transferred to a sample bottle and kept for further work.

2.2. Preparation of the coupon

Coupons of dimension 2 cm x 1.9 cm x 0.1 cm were prepared from mild steel rods purchased from the open market in Makurdi, and a tiny hole was drilled at the edge of each for suspension. After polishing to mirror finish the coupons using sandpaper, the coupons were degreased in acetone, and preserved in a desiccator. Subsequently, the initial weights (W_i) of the coupons were then made ready for corrosion studies. [15-19]

2.3. Corrosion studies

A 0.5 M solution of H_2SO_4 was prepared which served as the corrodent. Thereafter, the weighed coupons were placed in various concentrations of silver nanoparticles (0, 1, 2, 3, 4, 5) gdm^{-3} in 50 ml of the 0.5 M H_2SO_4. The corrodent with/without the inhibitor and coupons were placed in the thermostatic water bath set at 301 K for 6 hours. After the time interval, the coupons were removed, quenched in saturated ammonium acetate solution, washed in distilled water, and dried in acetone, kept in a desiccator, and then the final weight (W_f) was taken, thereafter. The procedure was repeated at 305 K, 309 K, and 313 K.

2.4. Weight loss measurement

The weight loss, inhibition efficiency (%IE), and corrosion rate (CR) was calculated using equations (1), (2) and (3), respectively as reported by different scholars. [6-10]

$$WL = W_I - W_F \tag{1}$$

$$\%IE = \left[1 - \frac{W_1}{W_2}\right] \times 100 \tag{2}$$

$$CR\ (mgcm^{-2}h^{-1}) = \frac{WL}{At} \tag{3}$$

where WL is the weight loss of the coupon, W_I is the weight before insertion, and W_F is the weight after retrieval, w_1 and w_2 are the weight losses (in grams) of mild steel coupon in the presence and absence of the inhibitor in the acid solution, WL is the weight loss in milligrams (mg), A the coupon surface area in cm^2, and t is the immersion time in hours. Equations (4) to (7) are used for the evaluation of activation energy, the heat of adsorption, enthalpy of activation and entropy, and Gibb's free energy, respectively.

$$logCR = logA - \frac{E_a}{2.303RT} \tag{4}$$

$$log\left(\frac{\theta}{1-\theta}\right) = LogA + LogK - \frac{Q_{ads}}{2.303R}\left(\frac{1}{T}\right) \quad (5)$$

$$ln\left(\frac{CR}{T}\right) = \ln\left(\frac{R}{Nh}\right) + \frac{\Delta S^*}{R} - \frac{\Delta H^*}{RT} \quad (6)$$

$$\Delta G_{ads} = -RT\ln(55.5\,K) \quad (7)$$

where K= $\frac{\theta}{(1-\theta)c}$, C is the concentration of the extract and 55.5 is the concentration of water expressed in moles. [24]

Langmuir, Freundlich, Temkin, and Adejo-Ekwenchi, adsorption isotherms (Equation 9-12 were used to model the adsorption process.

Langmuir $\frac{C}{\theta} = \frac{1}{K} + C$ (9)

Temkin $\frac{-2\alpha\theta}{2.303} = logK + logC$ (10)

Freundlich $log\theta = logK + nlogC$ (11)

Adejo-Ekwenchi $log\frac{1}{(1-\theta)} = logK_{AE} + blogC$ (12)

where K is the equilibrium constant, C is the concentration of the inhibitor, and n is a constant that tells the intensity of the adsorption process and has a typical value of 0.6 [24].

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1 Characterization of Sample

In this study ultraviolet (UV) and Fourier-transform infrared (FTIR) spectroscopy were used in the context of our research objectives. UV spectroscopy, widely employed for identifying and quantifying chemical compounds, operates by measuring the absorption of UV light by molecules, which provides insights into electronic transitions, conjugation, and the structural features of organic and inorganic substances. FTIR spectroscopy, on the other hand, is a powerful analytical tool for characterizing molecular vibrations, yielding detailed information about functional groups and molecular interactions. Together, these spectroscopic techniques allow for a comprehensive analysis of the compounds under study, enhancing our understanding of their photochemical and structural properties. Below are the specific results obtained from UV and FTIR spectra as well as corrosion studies. The discussions highlight the significance of the UV and FTIR spectra in the characterization process and the effect of silver sulfide (Ag_2SNP) using *Musa paradisiacal* peels in mitigating corrosion of mild still

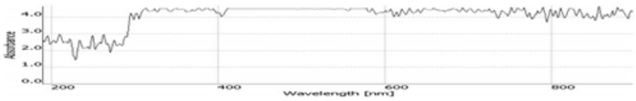


Fig 1: UV-vis spectral AgSNPs from *Musa paradisiaca* peels extract

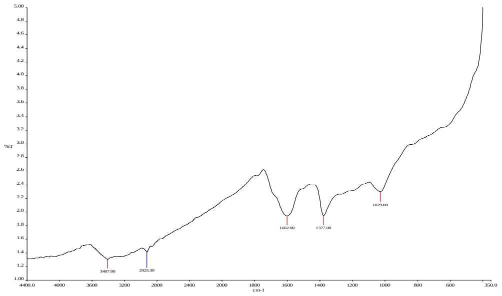


Fig 2: FT-IR spectral of AgSNPs from *Musa paradisiaca peels* extract

3.1.2. Corrosion studies

(a). Effect of Inhibitor Concentration

The values of weight losses (WL), inhibition efficiency (%IE), Surface coverage, and corrosion rate for the inhibition of mild steel corrosion by silver sulfide nanoparticles of *Musa paradisiacal peels* extract 0.5 M sulphuric acid at various concentrations and temperatures are presented in Table1 and 2, respectively.

Table 1: Weight Loss and Inhibition Efficiency for silver sulfide nanoparticle of *Musa paradisiacal peels* extract

Concentration	**WL (g)**				**%IE**			
(g/dm^3)	**301K**	**305K**	**309K**	**313K**	**301K**	**305K**	**309K**	**313K**
Blank	0.0548	0.0836	0.1159	0.1678				
0.1	0.0393	0.0564	0.0735	0.0989	28.2847	32.5359	36.5833	41.0608
0.2	0.0370	0.0489	0.0628	0.0878	32.4818	41.5072	45.8154	47.6758
0.3	0.0340	0.0374	0.0456	0.0629	37.9562	55.2632	60.6557	62.5149
0.4	0.0292	0.0309	0.0407	0.0531	46.7153	63.0383	64.8835	68.3552
0.5	0.0230	0.0249	0.0340	0.0389	58.0292	70.2153	70.6644	76.8176

Table 2: Corrosion rate for silver sulfide nanoparticle of *Musa paradisiaca*l peels

Concentration (g/dm^3)	301K	305K	CR 309K	313K
Blank	12.6852	19.3519	26.8287	38.8426
0.1	9.0972	13.0556	17.0139	22.8935
0.2	8.5648	11.3194	14.5370	20.3241
0.3	7.8704	8.6574	10.5556	15.9491
0.4	6.7593	7.1528	9.4213	12.2917
0.5	5.3241	5.7639	7.8704	9.0046

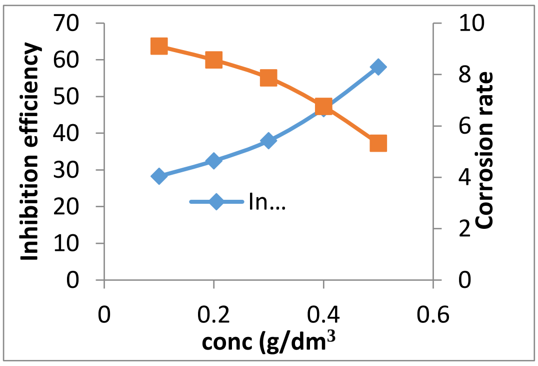


Figure 1: Variation CR and %IE at 301K.

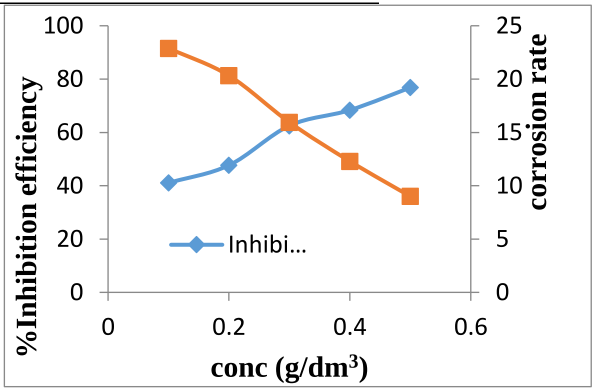


Figure 2: CR and %IE at 313K.

(b). Effect of temperature

The effect of temperature on the corrosion behavior of steel in 0.5 M H_2SO_4 containing AgSNPs from *Musa paradisiaca*l peels 0.1 -0.5 g/dm^3 is studied in the temperature range of 301-313 K using weight loss measurements for 6 hrs. The data of corrosion rates (W) and corresponding inhibition efficiency (%IE) collected were presented in Tables 1 and 2 above. Figures 3 and 4 show the effect of temperature on the corrosion behavior of mild steel in 0.5 M H_2SO_4 containing AgSNPs from *Musa paradisiaca*l peels at o.1 and 0.5 g/dm^3 only.

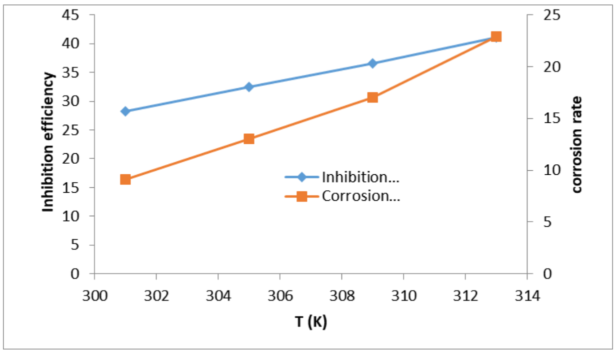


Figure 3: Variation of CR and %IE in 0.1g/dm^3 at different temperatures

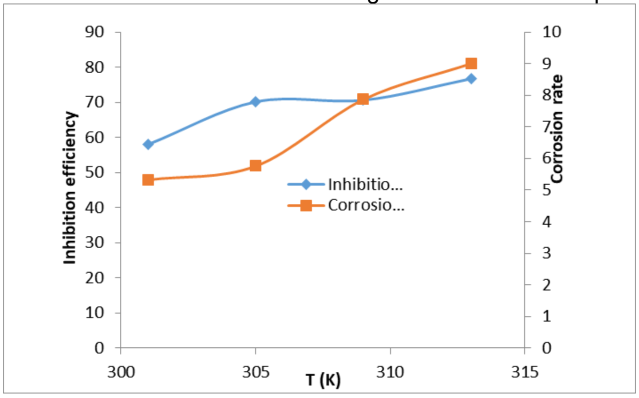


Figure 4: Variation of CR) and %IE in 0.5g/dm^3 at different temperatures

Table 3: Values of activation energy and thermodynamic parameters for silver sulfide nanoparticle of *Musa paradisiacal* peels as inhibitor for mild steel corrosion.

Conc. (g/dm^3	$E_{a.}$ (kJ/mol)	$+\Delta H^*$ ads (kJ/mol)	$+\Delta S^*$ ads (kJ/mol)	$+Q_{ads}$ (kJ/mol)	-ΔG ads (kJ/mol)			
					301 K	305 K	309 K	313 K
Blank	70.94	68.42	386.45	-				
0.1	58.37	55.85	341.97	80.09	13.49	14.18	14.82	15.51
0.2	54.96	52.44	329.83	39.67	12.25	13.40	14.02	14.40
0.3	45.42	42.90	296.91	51.93	11.84	13.78	14.52	14.53
0.4	40.25	37.73	278.53	52.85	12.02	13.86	14.25	14.84
0.5	36.32	33.81	263.84	50.84	12.60	14.12	14.36	15.38

(c). Effects of adsorption parameters

In the present study, the following adsorption isotherms were used to model the adsorption process of the inhibitor: Langmuir, Freundlich, Temkin, and Adejo Ekwenchi, isotherms.

Table 4: The Adsorption Parameters of Adsorption Isotherms

Langmuir	R^2	Kad	Constant	$-\Delta G_{ads}$
301K	0.850	0.7962		9.4823
305K	0.964	1.0395		10.2847
309K	0.982	0.9542		10.1996
313K	0.959	1.0040		10.4640
Freundlich			n	

301K	0.896	0.6998	0.426	9.1594
305K	0.985	0.9817	0.492	10.1396
309K	0.979	0.9616	0.425	10.2194
313K	0.960	0.9727	0.395	10.3816
Temkin			α	
301K	0.830	4.3251	-0.4514	13.7183
305K	0.962	6.9984	-0.6318	15.1211
309K	0.969	7.0958	-0.5792	15.3550
313K	0.928	7.6384	-0.5873	15.7455
Adejo Ekwenchi			b	
301K	0.777	2.5586	0.297	12.4044
305K	0.916	4.2858	0.495	13.8773
309K	0.948	4.4978	0.483	14.1835
313K	0.863	5.3703	0.553	14.8286

3.2. Discussion

3.2.1. Synthesis and characterization

A simple method using plant extract reduction has been developed for synthesizing silver nanoparticles, which could also be used for the synthesis of several metallic nanoparticles involving other metals with good size and shape morphology. [25] The results related to the metallic silver sulfide nanoparticles indicated the reduction of silver ions by *Musa paradisiaca* peels. Therefore, it can be concluded that the non-active or stationary cells of *Musa paradisiaca* peels can reduce silver ions in their periplasmic space. Initially, the synthesis of silver sulfide nanoparticles was confirmed by observing the color change of the reaction mixture [26, 27]. The appearance of a suspended gray-black color at room temperature suggested the formation of silver sulfide nanoparticles. The confirmation of formation and stability of the silver sulfide nanoparticles in the colloidal solution was monitored by using UV-Vis spectral analysis and FTIR. Ultraviolet-visible (UV-vis) spectra from 200 to 700 nm were measured using a Shimadzu UV-vis spectrophotometer (UV-3600, Japan). Silver sulfide nanoparticles usually exhibit strong absorbance in the range of 200-600 nm revealing good photo absorption properties. In Fig 1 above the spectrum revealed that the synthesized silver sulfide nanoparticles exhibit a powerful emission peak at 400 nm characteristic of Ag_2SNPs nanoparticle, due to its surface Plasmon resonance absorption band. [26, 27]

FTIR measurements were carried out to identify the possible biomolecules responsible for the reduction of the silver ions, and capping of the bio-reduced silver sulfide nanoparticles synthesized by *Musa paradisiaca* peels extract filtrate. Representative spectra of nanoparticles obtained from FTIR measurements manifested absorption peaks located at about 3407.00, 2925.30, 1602.00, 1377.00, and 1029.00 cm^{-1}. The FTIR spectra revealed the presence of different functional groups like the alcohol/phenol –OH stretching vibration, carboxylic acid –OH stretch, and N-H stretching of amides. The strong peak at 1620 cm^{-1} is characterized by alkene. It can be deduced that the flavonoids and terpenoids, which are abundant in *Musa paradisiaca*, show characteristic absorption peaks that appear to be responsible for the accelerated reduction and capping process, which give rise to the well-known signatures in the infrared region of the electromagnetic spectrum (Fig. 2). [28]

3.2.2. Effect of concentration and temperature

The effect of concentration and temperature on the corrosion of mild steel in 0.5 M sulphuric acid using silver sulfide nanoparticles synthesized from *Musa paradisiaca* peel extract as an inhibitor was investigated and the results are presented in Tables 1 and 2, respectively. Table 1 shows the values of weight loss and percentage inhibition efficiency of the inhibitor at various concentrations in an acid solution. The reduction in weight loss on the introduction of the nanoparticles into the corrodent is an indication that the silver sulfide nanoparticles of *Musa paradisiaca* have an inhibitive effect. [29] This percentage inhibition efficiency was observed to increase with the increase in the concentration of synthesized silver sulfide nanoparticles of *Musa paradisiaca*l peel extract with an increase in temperature (Fig. 1 and 2 at 301K and 313K). The highest inhibition efficiency of 76.8176% was obtained at 0.5 g dm^{-3} at 313 K and the least is 28.28 % at 301K for 0.1dm^{-3} concentrations. There is a significant difference between the values of % I. E with temperature rise which is suggestive of chemical adsorption mechanism (Fig. 3 and 4 at 0.1 g/dm^3 and 0.5 g/dm^3, respectively). [29]

Table 2 shows the surface coverage and corrosion rate for the inhibition of mild steel corrosion by silver sulfide nanoparticles of *Musa paradisiaca*l peels at various concentrations and temperatures. The corrosion rate which was observed to be high in the blank, significantly reduced as seen in the corrosion rate upon introduction of the inhibitor into the corroding medium. This indicates that the synthesized silver sulfide nanoparticles of *Musa paradisiaca*l peel extracts can be effectively used to mitigate the rate of mild steel in the acid medium. It was observed that the corrosion rate of the mild steel decreased with an increase in concentration and increased with an increment in temperature (Fig. 1, 2, 3, and 4]. This behavior explains the fact that the extent of adsorption and the coverage of inhibitor on mild steel surface increases with inhibitor concentration. [30]

This outcome demonstrates the capacity of Musa paradisiaca-derived nanoparticles to reduce the corrosion rate in an acidic environment. Similarly, studies by Zhang et al. (2021) on green inhibitors derived from plant extracts have shown that these natural inhibitors effectively reduce corrosion rates by forming a protective film on the steel surface, especially when used in higher concentrations. [31]

Furthermore, the corrosion rate inversely correlates with inhibitor concentration but directly correlates with temperature increases, as observed in this study’s results (Fig. 1, 2, 3, and 4). This trend is consistent with findings in similar research, where higher temperatures weaken the adsorption bond between the inhibitor molecules and the metal surface, reducing its effectiveness at elevated temperatures. [32] The observed decline in corrosion rates with higher inhibitor concentrations aligns with findings in related research on plant-based nanoparticle inhibitors, where the extent of adsorption and surface coverage on mild steel increased with inhibitor concentration, leading to more efficient protection. [33]

3.2.3. Activation energy and thermodynamic parameters for the inhibition process

The activation and thermodynamic parameters for the inhibition process for the nanoparticles are presented in Table 3. Computed values of activation energy values are all positive and significantly lower than those of the blank as shown in Table 3. Lower values of $E_{a.}$ in the presence of an inhibitor than in the absence of it suggest that the process is chemisorption while the reverse (higher) is an indication of physisorption. [24, 34] The enthalpy of activation (ΔH^*_{ads})which is a measure of the height of the energy barrier that has to be overcome by the reactant to attain a transition state. Computed values of enthalpy of activation are all positive and lower than that of the blank as shown in Table 3 indicating that the efficiency of inhibition increased with an increase in temperature. [35, 36] The dissolution process was also endothermic as the values were all positive. The average difference of $E_{a.} – \Delta H^*$ was found to be 2.52 kJolmol^{-1} [approximately equal to 2.55 kJmol^{-1} which is the value of RT (R is the universal gas constant and T is the average of the temperature at which the studies were conducted)]. This implies that the corrosion process of the metal in a medium is an unimolecular reaction.[37] The entropy of activation(ΔS^*_{ads}) values for the inhibitor are given in Table 3. The shift towards the positive value of entropies (S*) implies that the activated Complex in the rate-determining step represents dissociation rather than association, meaning that disorderliness increases on going from reactants to the activated complex.[38, 39]

The heat of adsorption (Q_{ads}) for all inhibitors were all positive indicating that the processes were endothermic. This means absorption of energy from the surroundings was required for the reaction to be sustained. [40]

The values of Gibbs free energy $(\Delta G_{ads})$for adsorption in aqueous solution are usually around -20 kJ mol^{-1} or lower (more positive) which indicate that the adsorption is due to electrostatic interaction between the inhibitor and metal (physisorption). While those around or higher (more negative) than -40 kJ mol^{-1} involve charge sharing or transfer of electrons between the molecules and metal (chemisorption). [41, 42] The negative ΔGads values in Table 3 indicate that adsorption is spontaneous, meaning it occurs naturally without external energy input. As the temperature increases, these values become less negative, suggesting that while the process remains spontaneous, its driving force diminishes with rising temperature. The stability of the adsorption values being below or slightly above -20 kJ mol^{-1} further signifies that the adsorption mechanism is likely physical adsorption (physisorption), which involves weaker interactions compared to chemical The negative (ΔG_{ads})values in Table 3 suggest that the adsorption process is spontaneous, aligning with similar research findings (Hassan & Mohamed, 2018)., [43 44] where an increase in temperature resulted in less negative (ΔG_{ads}) values, indicating a decrease in the spontaneity or driving force for adsorption.

As observed in prior studies, the lower magnitude of (ΔG_{ads}) , often below -20 kJ mol^{-1}, supports the conclusion that the adsorption mechanism is physisorption. This process is typically reversible and relies on weaker forces, unlike chemisorption, which is usually stronger and less reversible (Chen et al., 2021). [45] This trend aligns with the work of Hassan and Mohamed (2018) [44], who noted a similar reduction in spontaneity with temperature, suggesting that increased thermal energy diminishes the attraction between the inhibitor and metal surface.

3.2.4. Adsorption isotherm for the inhibition process

The mechanism and action of inhibition have been attributed to the adsorption process of the adsorbate (inhibitor) onto the surface of the metal (adsorbent); hence adsorption isotherm models have been extensively used in its confirmation. In the present study, the data obtained from weight loss method were fitted into the various two-parameter isotherms. Going by the coefficient of determination (R^2) the adsorption process can be said to fit into the Langmuir, Freundlich, Temkin, and Adejo Ekwenchi isotherms.

(a) **Langmuir Isotherm:** Langmuir isotherm adsorption is usually an indication of monolayer coverage of inhibitor on the surface of mild steel. The slopes and intercepts are given in Table 4, along with the coefficient of determination (R^2) for nanoparticles of silver sulfide synthesized from *Musa paradisiaca*l peel extracts. An ideal Langmuir isotherm plot should have a good R^2 value (unity) and intercept of zero (Fig. 5a) with a positive adsorption equilibrium constant K [46]**.** In reference to the values of regression coefficient R^2 as shown in Table 4, the adsorption equilibrium constant K values are positive and intercept close to zero indicative of the suitability of the Langmuir isotherm to the adsorption behavior of inhibitors. Langmuir isotherm applies to both physisorption and chemisorption. Hence Langmuir isotherm can be used to model the adsorption of this study.

(b) **Temkin Isotherm:** The Isotherm constant and coefficient of determination of (R^2) are presented in Table 4 and Fig. 5b. The constant α which is related to the heat of adsorption, equally increases with an increase in temperature, which is a characteristic of chemisorption. Examination of the data shows that the Temkin isotherm applies to the inhibitor adsorption on mild steel and the adherence of this adsorption layer. The negative values of “α” are indications of repulsive interaction in the absorbed layer, the increase in the value of ΔG_{ads} with temperature rise is a feature of chemical adsorption. [40]

(c) **Freundlich Isotherm:** The Freundlich constant has to do with the adsorption intensity and the heterogeneity of the material, and its good value should be close to 0.6. [47] The values of the parameter n_f obtained were not close to 0.6 The fact that the obtained average value of n (0.4345) is not close to 0.6 implies that this adsorption process cannot be modeled by this isotherm. [48, 49] despite having a good value of regression coefficient (R^2)

(d) **Adejo Ekwenchi Isotherm:** Values of regression coefficient (R^2) of Adejo-Ekwenchi Isotherm can be seen in Table 4 and Fig. 5d. Adejo-Ekwenchi Isotherm as it seems to be obeyed by nearly any adsorption process, is centered on the fact that for any adsorption process, the available surface of a given quantity of the adsorbent decreases with increase in the concentration of the adsorbate. Hence the difference between the total available surface on the adsorbent and the fractional surface coverage decreases with an increase in the adsorbate concentration, that is, the more the surface coverage the less the available surface. Therefore, there is an inverse relationship between the available surface and the concentration of the adsorbate. [35] A decrease in the b value with temperature rise signifies physisorption, while an increase or fairly constant value indicates chemisorption. From Table 4, it is obvious that the absorption of the inhibitors onto the metal surface is chemisorption, as b increases with temperature or is fairly constant.

4. CONCLUSION

The results related to the metallic silver sulfide nanoparticles indicate the reduction of silver ions by *Musa paradisiaca* peels. Therefore, it can be concluded that the resting cells of *Musa paradisiaca* peels can reduce silver ions in their periplasmic space. The confirmation of formation and stability of the silver sulfide nanoparticles in the colloidal solution was monitored by using UV-Vis spectral analysis and FTIR. From the above results, it has been shown that silver sulfide nanoparticles of *Musa paradisiaca* peels extract are a good inhibitor that can be used when developed to mitigate the rate of corrosion since it is environmentally benign as this will greatly enhance global sustainability.

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BOOK OF PROCEEDINGS
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Adsorption of Rhodamine-B on Functionalized Hydroxyapatite-Chitosan-Titanium Dioxide Nanoparticles

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ABSTRACT

The research connotes the beneficial use of decomposable Functionalized Hydroxyapatite-Chitosan-Titanium Dioxide Nanoparticles (FHC-TiO_2NPs). Hydroxyapatite produced from the Periwinkle shell was functionalized with Chitosan and TiO_2NPs for uptake of Rhodamine B (Rh-B) from aqueous medium. The adsorbent was characterized by Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction Analysis (XRD), Scanning Electron Microscopy (SEM), and Energy Dispersive X-ray Spectroscopy (EDX). Reduced strengths of peaks, greater irregular cavities, and lesser particle diameters of functionalized biogenic materials confirmed functionalization. The adsorption efficiency was obtained at pH 9 (26.38 %), dosage 0.5 g (91.51 %), initial Rh-B concentrations 10 mg/l (95.23 %), contact time 10 min (2.82 mg/g), and temperature 303 K (52.29 %). The batch adsorption revealed that FHC-TiO_2NPs are capable and sustainable sorbent for eliminating Rh-B dye from aqueous medium due to its constant availability.

KEYWORDS: Adsorption, Rhodamine B, Biosorbent, Functionalization, Nanoparticles, Characterization.

1. INTRODUCTION

Rh-B is a glowing dye usually utilized in various scientific and industrialized applications. At the same time, nanoparticles (NPs) have added substantial care for their exclusive properties and potential applications across different fields.[1] Rh-B is frequently employed as a fluorescent probe to label biological samples, visualize cells and track molecular interactions in various experiments. Additionally, it finds applications in the textile, cosmetics, and food industries as a colorant.[2]

Hydroxyapatite is a crystalline substance that belongs to the apatite group of minerals and has a chemical formula of $Ca_5(PO_4)_3OH$.[3] This unique composition gives hydroxyapatite its exceptional biocompatibility and makes it an essential component of the skeletal structure. Hydroxyapatite forms elongated crystals that are similar in shape to needles, with a white to off-white color.[4] Hydroxyapatite nanoparticles are utilized in the development of advanced drug distribution systems and material engineering scaffolds.[5] Nanoparticles can be engineered to possess specific properties, making them valuable for targeted drug delivery, imaging, sensing, and enhancing material performance.[6]

Chitosan-titanium dioxide nanoparticles (CTNPs) are composite materials formed by combining chitosan, a biopolymer derived from crustacean shells, with TiO_2NPs.[7] Continued research and development in this field will likely unveil new opportunities for CTNPs in diverse industries, contributing to technological advancements and improved sustainability.[8] The aim of this research is to examine the batch adsorption performance of Rh-B on FHC-TiO_2NPs.

2. MATERIALS AND METHODS

2.1. Materials

Titanium dioxide (TiO_2), Hydrochloric (HCl), Sodium hydroxide (NaOH), Sodium chloride (NaCl), and Rh-B with molecular formula $C_{28}H_{31}N_2O_3Cl$ was bought from Sigma-Aldrich, Germany were all used to model dye wastewater by making a stock solution of 0.1 gl^{-1} Rh-B. The chemicals used in these studies were obtained from Sigma Aldrich. Fresh periwinkle shells were obtained from Oyingbo market, Lagos State. All reagents and chemicals used were of analytical grade.

2.2. Production of Hydroxyapatite from periwinkle shell.

The dried up periwinkle shell (PS) was calcined using an electronic furnace at 600 °C for 2 hrs. The calcined sample was crushed into granular form and sieved to a unit size of 2 mm (to allow higher compaction for improvement of adsorptive property), soaked with 0.26M Di-ammonium hydrogen phosphate solution (acts as a precursor for the formation hydroxyapatite) for 24 hrs. The liquid part was decanted and the sediment was wash away continually with distilled-deionized water and drained using a Buchner funnel and suction pump, this was then oven-dried at 105 °C for 2 hr to eliminate moisture content completely. Prepared PSHAP was reserved in a tight container and kept in a desiccator for further use.[9,10]

2.3. Preparation of Adsorbent and Characterization

The hydroxyapatite and chitosan as adsorbents for the adsorption process were functionalized using TiO_2NPs. 20 ml of TiO_2NPs was measured into a 100 mL beaker and 40 g of Hydroxyapatite and chitosan were weighed and added to it. The mixture is stirred thoroughly for some minutes. After the mixture was thoroughly stirred, it was placed inside the oven- and dried at 60^0C for hours.

Surface chemistry of hydroxyapatite and chitosan with TiO_2NPs was characterized by FTIR Model 500, Buck Scientific Inc.) in the range of 400– 4000/cm, SEM (Bruker-X Flash 6130, Carl Zeiss, EVO 18 Research, Germany) in the wavelength λ = 10 – 10m, EDX (JEOL JSM-6510LA, Japan).[11,12]

2.4. Preparation of Adsorbate Solution

The stock solution using 1000ml of the adsorbate was arranged by adding 0.2 g of Rh-B to 1000 ml of distilled water. Subsequently, 50 ppm was prepared from the stock solution.[11,12]

2.5. Adsorption Studies

The pH point of zero charges (pHpzc) was determined. A considered amount (0.25g) of hydroxyapatite-chitosan-TiO_2NPs was weighed and put into 50 ml of 0.1 M NaCl with a prearranged pH in a 250-ml conical flask. The pH of the resulting solution was modified between pH 1 and 12 with either 0.1 M NaOH or HCl. The conical flasks were enclosed and left for 24 hrs after which the last pH values were determined. The variance between the initial and final pH was calculated and plotted against the initial pH.[11,12,10]

Effect of pH was studied between pH 2 and 12 by adjusting Rh-B solution with 0.1 M HCl or 0.1 M NaOH, it was decanted and centrifuged at 3000 rpm for 15mins followed by the measurements of its absorbance spectrum using UV-Visible spectrophotometer operated at the range 190-900 nm.

The effect of dosage was investigated. Approximately 0.1 to 0.5 g of adsorbent (hydroxyapatite-chitosan-TiO_2NPs) was added to 50 ml of 50 mg/L Rh-B solution in a 250 ml conical flask respectively. The flasks were agitated at 300 rpm for 30 min in a thermostat-regulated water bath with a shaker (Uniscope water bath shaker) at 30℃, It was decanted and centrifuged at 3000rpm for 15min, followed by the measurements of its absorbance spectrum using UV-Visible spectrophotometer operated at the range 190-900 nm.[11,12,10]

The effect of initial concentration of Rh-B was investigated. Rh-B solution was diluted through serial dilution used to prepare the other five concentrations (10-50 mg/l). Exactly 1 g of hydroxyapatite-chitosan-TiO_2NPs was added to 100 ml of each solution and put inside the water bath shaker agitated at 150 rpm at an ambient temperature condition of 30 ℃. Then Rh-B solution solution was decanted and centrifuged at 3000 rpm for 15 min.

The effect of temperature was investigated. 0.25g of the adsorbents was added to 50 ml of 50mg/l Rh-B solution in a 250ml conical flask, the mixture was put inside the shaker and then agitated at 150 rpm and 30^0C. The samples were taken after 30 mins of agitation. The influence of solution temperature on the Rh-B sorption process was observed at different temperatures: 35, 40, 45, 50, and 55°C respectively. This was done by adjusting the temperature regulator of the water bath shaker.[11,12,13]

The effect of contact time on Rh-B concentrations was investigated. 5g of hydroxyapatite-chitosan-titanium dioxide nanoparticles were weighed into 500 ml of Rh-B solutions separately, and 100 mins were used for the contact time. The water bath was adjusted to 30^0C, 5ml was taken from each of the solutions after 10-minute centrifuges, and a UV spectrometer was used to determine the absorbance.[11,12,13] Percentage removal and quantity adsorbed at a given time t were calculated using the following equations:

$\% \, Removal = (Co - C_f / \, Co) \times 100$ …………………………………… (1)
$qt = (Co - C_t) \, V/M$ ……………………………………………………………………. (2)
Where qt is the amount of Rh-B adsorbed per unit mass of adsorbent (mg/g)
Co is the initial Rh-B concentration (mg/l), C_f is the final Rh-B concentration (mg/l). , Ct is the residual concentration at time t, V is the volume of Rh-B solution (l) and M mass of adsorbent (g).

3. RESULTS AND DISCUSSION

The FTIR study was used to define the functional groups existing in the FHC-TiO_2NPs (figure 3a-c). The occurrence of peak at 3433.41 cm^{-1} was owing to the stretching vibration of the N-H bond in the chitosan. The peaks at 1743.88 and 1637.95 cm^{-1} are owed to the stretching vibrations of the C=O bond in the amine and amide groups of the chitosan, respectively. The peak at 1387.56 cm^{-1} was as a result of the bending vibration of the C-H bond in the amine group. The peaks at 1006.80 and 875.55 cm^{-1} were owing to the stretching vibrations of the P-O bond in the hydroxyapatite confirms that the nanoparticles had been successfully functionalized with hydroxyapatite and chitosan.

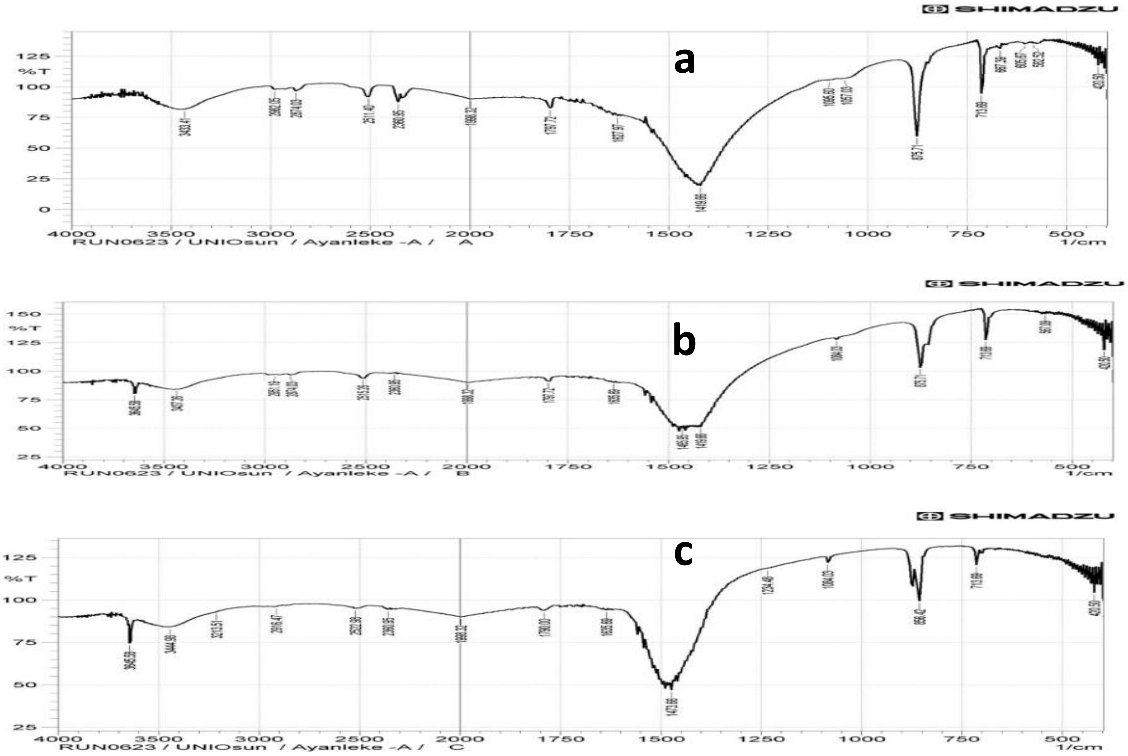


Figure 3a-c: FTIR spectra of (a) FHC-TiO_2NPs (b) Hydroxyapatite (c) Chitosan

The XRD pattern of the FHC-TiO_2NPs showed the characteristic peaks of both hydroxyapatite (HA) and chitosan (Figure 3d-f). This indicates that TiO_2NPs had been successfully functionalized with HA and chitosan.[11,12]

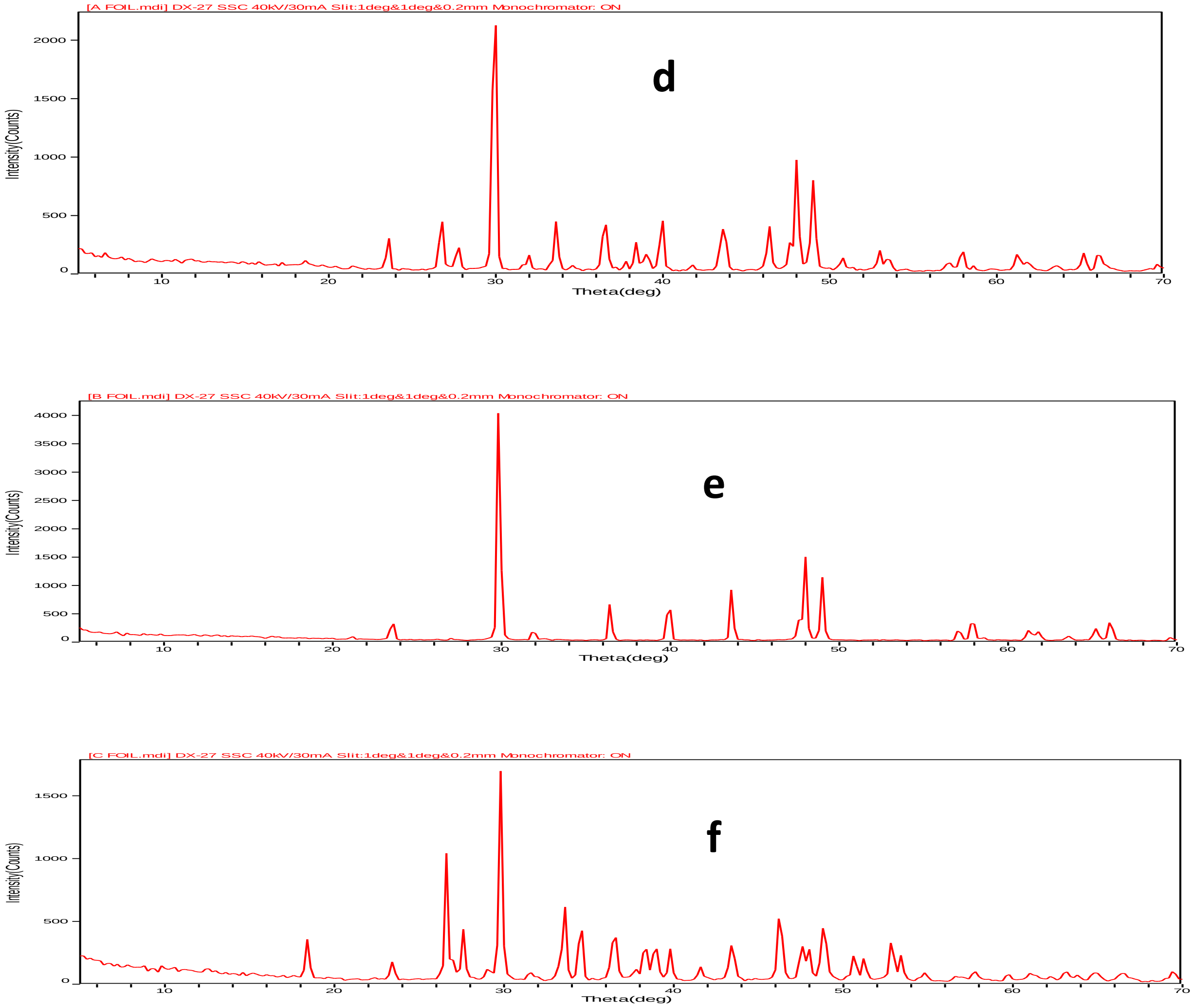


Figure 3d-f: XRD spectra of (d) FHC-TiO_2NPs (e) Hydroxyapatite (f) Chitosan

The SEM image (figure 3g) showed the surface of FHC-TiO_2NPs. The SEM image of the hydroxyapatite produced from the periwinkle shell (Figure 3h) showed that the particles are amorphous, meaning that they do not have a regular crystalline structure. The SEM image of chitosan produced from periwinkle shells (Figure 3i) showed that the molecules of chitosan are arranged in a hexagonal shape. The overall appearance of the SEM image is consistent with the expected properties of chitosan. [11,12]

Figure 3g: SEM image of FHC-TiO_2NPs

Figure 3h: SEM image of Hydroxyapatite

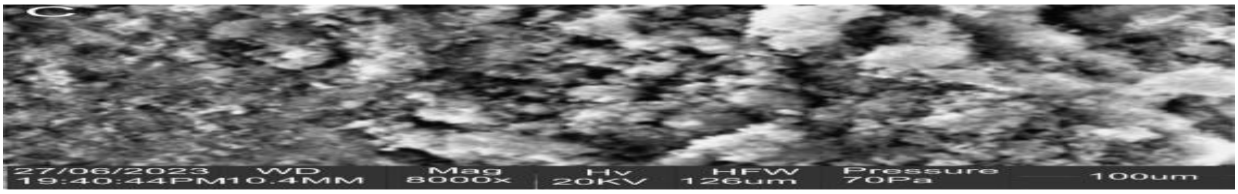
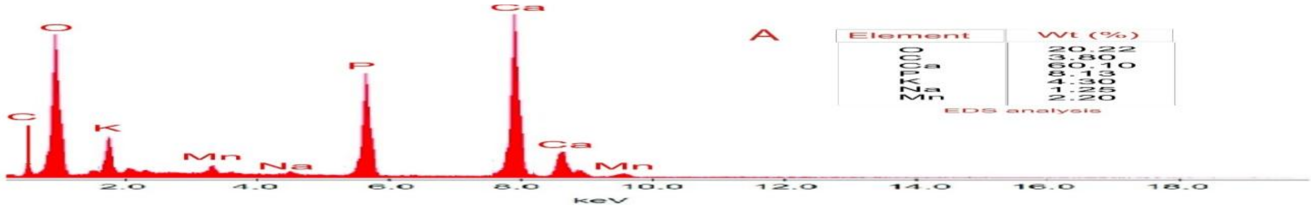


Figure 3i: SEM image of Chitosan

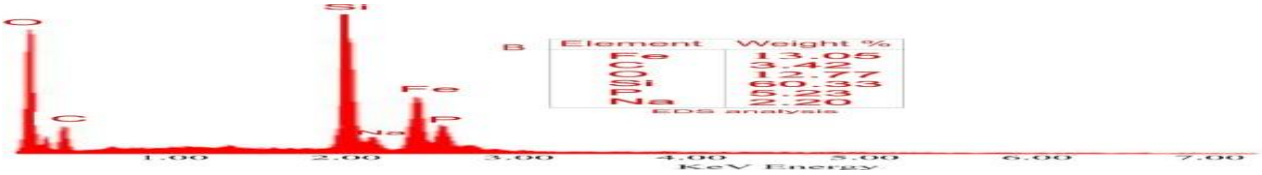
The EDX results showed FHC-TiO_2NPs were successfully functionalized (Fig. 3j). The major elements present are Calcium (60 %), Oxygen (20 %), Phosphorus (8 %), Potassium (4n %), Carbon (4 %), Manganese (2 %) and Sodium (1 %) (Azeez *et al*., 2022, 2024). EDX results (Fig. 3k) showed that hydroxyapatite produced from the periwinkle shell was made up of the following elements: Silicon (60 %), Phosphorus (13 %), Calcium (12 %), Iron (3 %), and Sodium (2 %). The EDX result of Chitosan produced from periwinkle shells (Fig. 3l) showed that the chitosan has a high concentration of calcium and magnesium. This is consistent with the fact that periwinkle shells are a good source of these minerals.[11,12]



Element	Wt (%)
O	20.22
C	3.80
Ca	60.10
P	8.13
K	4.30
Na	1.25
Mn	2.20

EDS analysis

Figure 3j: EDX of FHC-TiO_2NPs



Element	Weight %
Fe	13.05
C	3.42
O	12.77
Si	60.33
P	5.23
Na	2.20

EDS analysis

Figure 3k: EDX of Hydroxyapatite

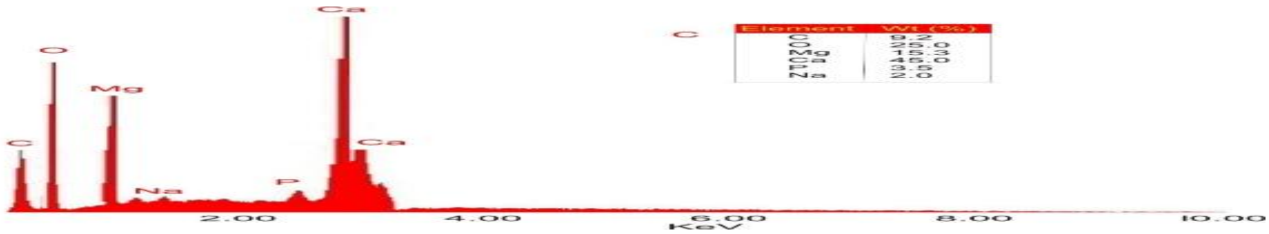


Figure 3l: EDX of Chitosan

3.1. Adsorption Studies

pHpzc for FHC-TiO_2NPs occurred at 9.0 suggesting maximum adsorption of Rh-B will be effective above this pH value (fig.3.1a).

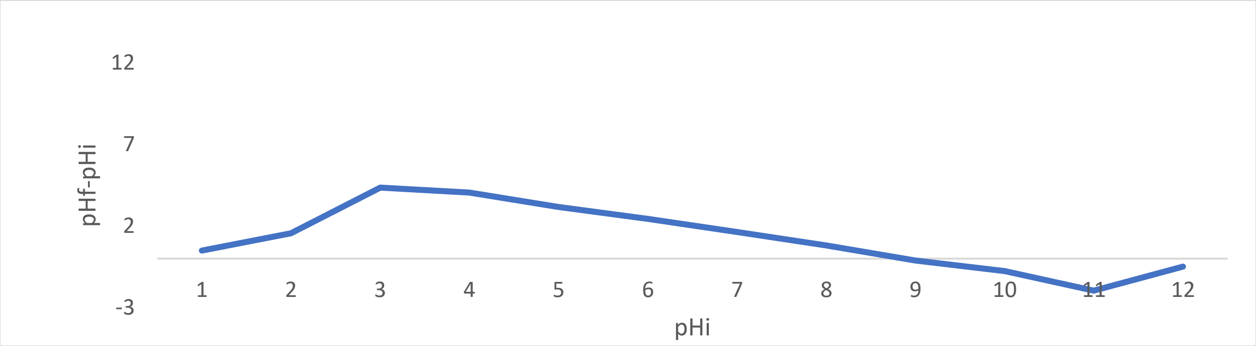


Fig. 3.1a: pH point of zero charge

The relationship between the initial pH value (2-12) and the percentage of Rh-B removal onto FHC-TiO_2NPs showed an irregular pattern. Therefore, the maximum adsorption percentage removal efficiency (26.38 %) was attained at a pH of 9 (fig.3.1b).

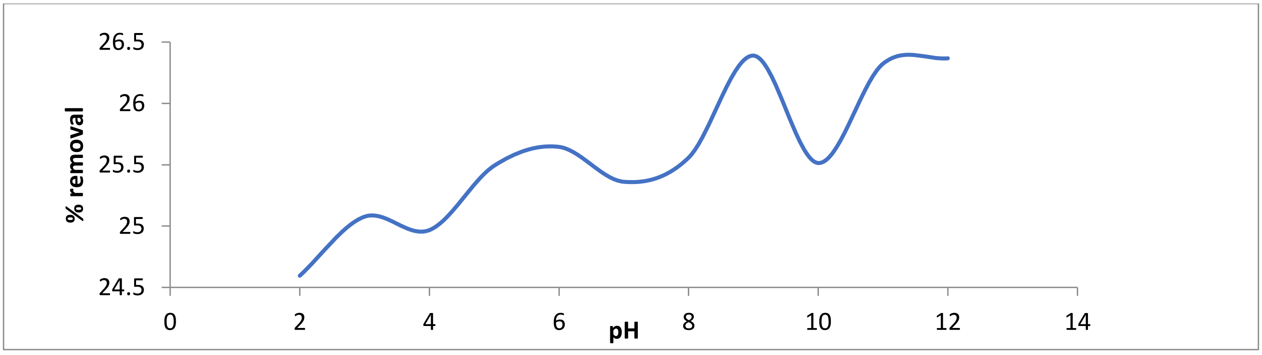


Fig.3.1b: Effect of pH on the adsorption of Rh-B

The outcome of the upshot of the adsorbent dosage on the percentage Rh-B removal efficiency (91.51 %) from aqueous medium was attained at 0.5 g. The percentage removal with an upsurge in adsorbent prescription from 0.1 to 0.5g for FHC-TiO_2NPs (fig.3.1c). This trend might be connected to a rise in the adsorbent surface area and accessibility of more vital sites on the surface of FHC-TiO_2NPs**.** The outcome of the upshot of Rh-B on the percentage removal of the Rh-B on FHC-TiO_2NPs.

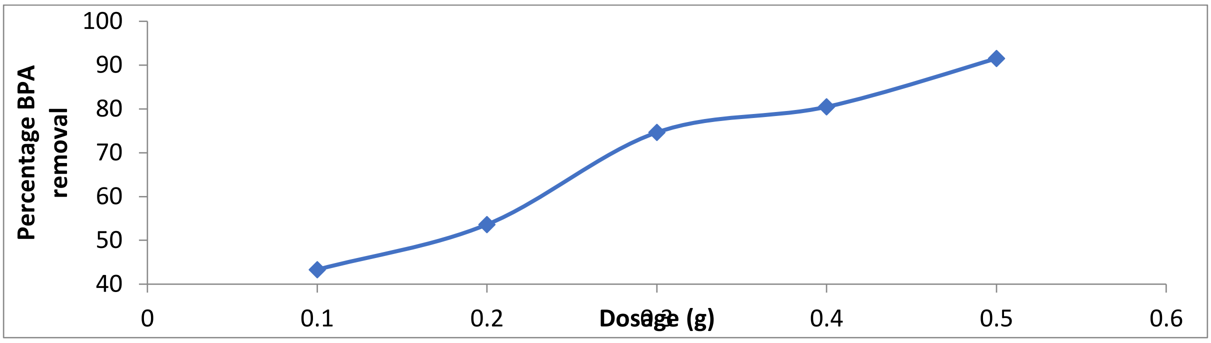


Fig.3.1c: Effect of adsorbent dosage

The percentage removal of Rh-B reduces with an upsurge in the initial Rh-B concentration, the extreme removal effectiveness obtained from Rh-B was 95% at 10 mg/l (fig.3.1d). The decrease in the removal efficiency of Rh-B as the initial increase suggested that the absorbent sites are filled up early in the process and maximum adsorption was achieved.[11,12,10]

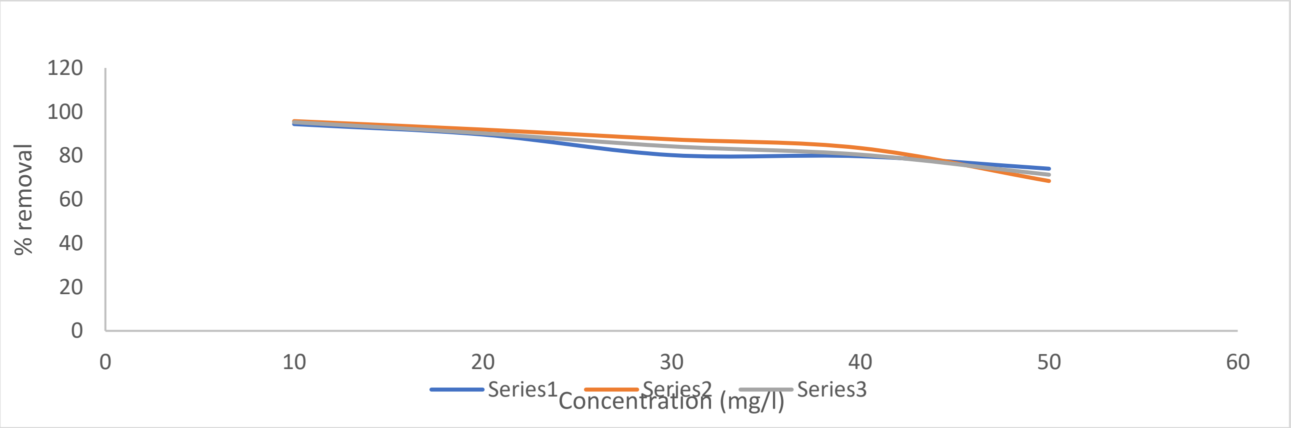


Fig 3.1d: Effect of initial conc. Rh-B

The outcome of the upshot of contact time on Rh-B uptake onto FHC-TiO_2NPs was presented (Fig 3.1e). The amount of Rh-B adsorbed at time t (qt) was initially rapid within the first 10 minutes and slowed steadily before attaining equilibrium at 60 minutes (2.82 mg/g). The initial upsurge in quantity adsorbed is attributed to the obtainable abundant surface reactive sites which were engaged as the adsorption proceeded.

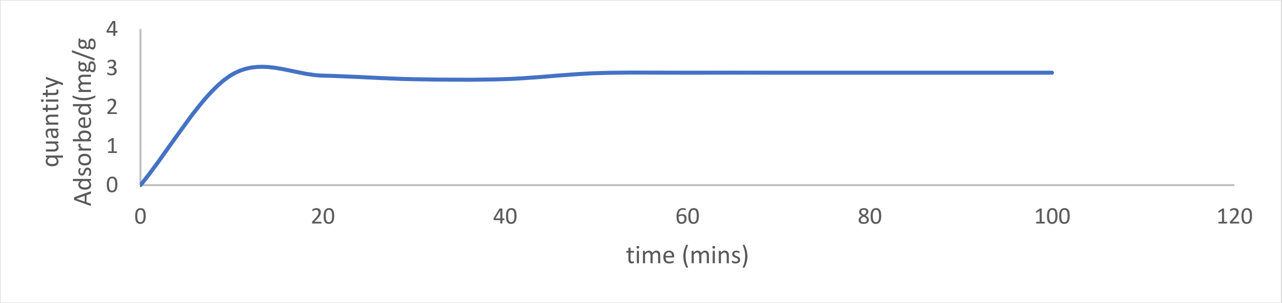


Fig.3.1e: Effect of contact time

The upshot of temperature on percentage Rh-B removal efficiency onto FHC-TiO_2NPs was 52.29 % at 303 K (Fig.3.1f). Removal efficiency of Rh-B decreased with a rise in temperature for the adsorbent used. The binding forces between the adsorbent and the adsorbate reduces with an upsurge in the temperature and subsequently upshot in reduced percentage removal.[11,12,10]

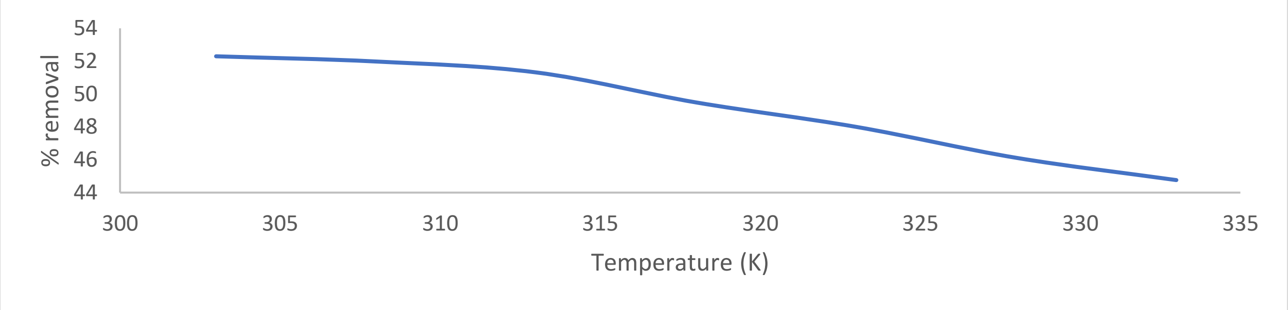


Fig 3.1f: Effect of temperature

4. CONCLUSION

This study showed that pH, dosage, initial concentration, temperature, and contact time affected surface functionalized hydroxyapatite-chitosan-TiO_2NPs, which improved its adsorptive performance and reusability. The uptake of Rh-B dye onto FHC-TiO_2NPs\ was greatly reliant on operational parameters. It also revealed that FHC-TiO_2NPs material is a capable and sustainable sorbent for eliminating Rh-B dye from aqueous solution due to its constant accessibility and reusability.

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BOOK OF PROCEEDINGS
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Determination of Organochlorine Pesticides of Soil in Arable Farmland of Joseph Sarwuan Tarka University, Makurdi, Benue State

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ABSTRACT

Six sub-soil samples from portions (A and B) were collected from designated sites in Joseph Sarwuan Tarka University commercial rice plot, analysed for pesticide residues and metabolites using gas chromatography-mass spectrometry. It was found that all detected organochlorine pesticides were lower than the US maximum residue limit of 0.03 mg/kg for agricultural soils. Higher concentrations were found in soil samples A, with Heptachlor, Aldrin, O-terphenyl and Dieldrin being present. O-terphenyl showed the highest concentration (mg/kg) of 0.01 in soil sample A and 0.0099 in soil sample B. Mean concentration (mg/kg) of pesticides detected in soil sample A shows that Heptachlor was 0.006±0.002, Aldrin (0.005±0.001), O-terphenyl (0.01±0.001) and Dieldrin (0.005±0.002). Heptachlor epoxide, Mirex, Endrin and Endosuulfan II were not detected. Physicochemical properties of soil samples A and B were similar. Presence of pesticides residues in samples A and B, suggests possibility of their continued use by farmers in the area despite restrictions in Nigeria. Sustainable remediation options to improve agricultural produce and reduce health threats remain a priority.

KEYWORDS: Pesticide residue, Organochlorine, Physicochemical, Soil, Extraction.

1. INTRODUCTION

Pesticide residues in food, crops are classified as organochlorines, organophosphates, carboxylic acids and their derivatives. Organochlorines (OCs) are synthetic, persistent chemicals with high molecular mass, moderate polarity and stability, used in agriculture and industry for pesticidal qualities. They have field half-life of 15-9 days.[1,2] Organochlorine pesticides (OCPs) are lipophilic, bioaccumulate in adipose tissues, alter nerve membranes of enzymes and pose environmental health risks.[3] Most OCPs have acute toxicity at concentrations higher than environmental limits. Daily cosmetic use with estrogenic activity exposes the skin, accumulates and harms internal organs.[4] OCP levels vary with age and consumption rates of contaminated products.[5] This is a follow-up study to that of Onuwa *et al.*[6] to check dessipation trends and its aim is to identify and quantify pesticide residues in soil within farmland in comparison with maximum residue limits (MRLs).

2. MATERIALS AND METHODS

2.1. Study Area

Soil samples were collected at Joseph Sarwuan Tarka (J.S. Tarka) University rice plot designated as sites 'A' further divided into two portions coded A and B for the two samples. The site was within latitude 7° 47' 0''N, longitude 8° 44' 40''E as shown in Figure 1.

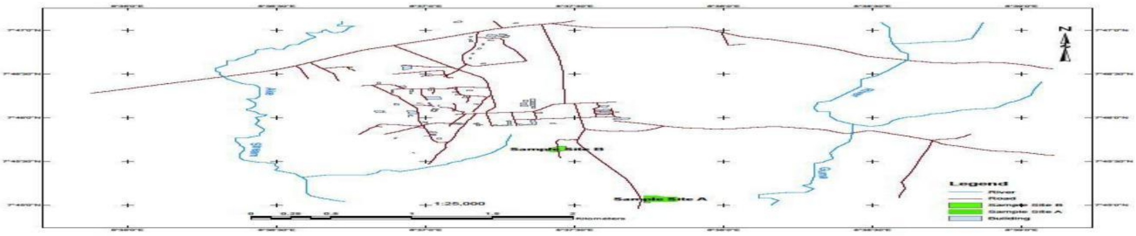

Figure 1: Map of South-Core, Joseph Sarwuan Tarka University Makurdi showing sampling Sites (Onuwa *et al.*, 2017).

2.2. Materials

Materials used were washed, rinsed with distilled water and dried. All reagents were of analytical grades. This includes H_2O, $Ca(OH)_2(aq)$, CH_3CN, NH_4OACl, $Na_2SO_4(s)$ and C_6H_{12} obtained from Thermo Fisher Scientific USA. Instrument/equipment were GC-MS (Agilent 6890N), Agilent 5975B mass selective detector, atomic absorption spectrophotometer (210VGP), centrifuge, flame photometer (FP-64), hydrometer-pulse splitless mode with an injection volume of µL, mechanical shaker and roller.

2.3. Sample Collection and preparation

Soil Samples (A and B) were collected from J.S Tarka University Makurdi rice plot according to standard method used by Ize-Iyamu *et al.*[7] . Two portions of soil samples were collected 132 m apart, using soil auger of 90 cm (claw of 20 cm). Six soil sub-samples were obtained, homogenized and bulked to form a composite, taken to laboratory in plastic bags. They were dried at room temperature for 6 days, then mechanically treated followed by soil analysis. They were ground, separated with 2 mm sieve, extracted using a soil-packed bulb column, 25 g of each sample was weighed into a glass jar, fortified, 25 g of pre-cleaned sand and 50 g of granular sodium sulphate were added. Mixture was manually shaken for 30 s, placed on a roller for 30 s, allowed to stand for 20 mins for absorption of residual moisture from the soil by Na_2SO_4. Mixture was transferred to a 250 mL bulb column; sample jar was rinsed three times with 5 mL hexane and transferred to the bulb column. Soil was extracted with 250 mL acetone-hexane mixture (1:1 v/v), the eluate collected, concentrated to 100 mL using rotary evaporator. Soil extract was cleaned-up to remove interference. Concentrated soil eluate was washed.

2.4. Soil Texture

This was carried out according to standard hydrometer method of mechanical analysis described APHA [8] and adopted with by Juo [9] using glass cylinder, hydrometers, thermometer and (5 %) sodium hexametaphosphate as dispersing agent. Soil pH was determined by a calibrated gas electrode pH meter to pH 7.0, 4.0 buffer before used. CEC was measured using centrifuge based on standard method by FAO.[10]

2.5. Extraction of Soil Samples

It was based on standard method described by Fosu-Mensah[11] and applied by Awe *et al.*[12] Soil sample (10 g) was weighed into 250 mL flask, 10 mL acetonitrile was added and ultra-sonicated for 5 mins. Flasks were closed with additional 10 mL acetonitrile, placed on a mechanical shaker for 30 mins at 300 M/Min and contents separated into layers after standing for 10 mins. Supernatant (10 mL) was taken, dried over 2 g $Na_2SO_{4(s)}$ through filter paper into 50 mL flask. Extract was reduced to 2 mL using rotary film evaporator,cleaned-up and passed to GC vials for analysis. Extracts were kept frozen.

2.6. Pesticide Residues Analysis and Recovery

OCPs in sample extracts were determined by 6890N gas chromatograph interfaced with 5975B mass selective detector (Agilent Technologies, Santa Clara, USA). ADB-5 capillary column (30 m length × 0.25 µm film thickness × 0.25 mm) was used for separation and helium gas (flow rate 1mL/min) used as carrier gas. The column had initial temperature of 70 °C, held for 20 mins, increased at 25 °C min^{-1} to 150 °C, 200 °C at 3 °C min^{-1}, then to 300 °C at 2 °C min^{-1}. Temperature of injection port, ion source, quadrupole and transfer lines were 250, 230,150 and 280 °C each. Sample was injected into the GC via a pulsed split less mode with an injection volume of 4µL. Quantification of pesticides was done using Mass Spectrometry Detector. Retention time, peak area and peak height of samples were compared with standards for detection of analytes. Method was optimized, validated using spiked and internal standards of required pesticide samples obtained from NIST to evaluate recoveries of OCPs which ranged 80-120 %. Procedure blanks and triplicate samples were analysed. Limit of detection was 0.001ppm.

3. RESULTS AND DISCUSSION

3.1. Physicochemical and organochlorine analysis of Soil

The results from the physicochemical and organochlorine analysis in the soil samples are given in Tables 1-3 and Figures 1 and 2.

Table 1: Mean concentration in (mg/kg) of Organochlorine pesticides in Soil Samples

Heptachlor	0.006 ± 0.002
Aldrin	0.005 ± 0.001
Heptchlor epoxide	ND
Mirex	ND
O-Terphenyl	0.01 ±0.001
Dieldrin	0.005 ± 0.002
Endrin	ND
Endosulfan 11	ND

ND: Not detected.

Table 2: Mean Physicochemical Properties of Soil Samples

Physicochemical Properties	Soil Samples	
	Sample A	Sample B
pH	5.65 ± 200.	6.19 ± 0.20
CEC (mol/kg)	6.88 ± 0.21	7.28 ± 0.18
Ca (mg/kg)	2.82 ± 0.08	2.92 ± 0.03
Mg (mg/kg)	2.60 ± 0.10	2.77 ± 0.08
K (mg/kg)	0.25 ± 0.03	0.28 ± 0.01
Na (mg/kg)	0.21 ± 0.01	0.25 ± 0.02
TEB (mol/kg)	5.88 ± 0.21	6.23 ± 0.12
Exchangeable Acid	1.00 ± 0.00	1.10 ± 0.00
Sand (%)	68.18 ± 0.07	69.78 ± 0.08
Silt (%)	12.17 ± 0.29	12.47 ± 0.50
Clay (%)	19.65 ± 0.27	17.75 ± 0.55
Texture Class	Sandy loam	Sandy loam

Table 3: Coefficient of Variation for Physicochemical Properties of Soil samples

Physicochemical Properties	**Soil Samples** **Sample A (%)**	**Sample B (%)**
pH	4	3
CEC (mol/kg)	3	3
Ca (mg/kg)	3	1
Mg (mg/kg)	4	3
K (mg/kg)	12	3
Na (mg/kg)	5	8
TEB (mol/kg)	4	2
Exchangeable Acid	0.0	0.0
Sand (%)	0.1	0.1
Silt (%)	2	4
Clay (%)	1	3

TEB: total exchangeable base, **CEC:** Cations exchangeable capacity.

3.2. Organochlorine Pesticide Residues in Soil Samples.

Table 1 shows mean concentration in (mg/kg) of OCPs in soil samples. O-Terphenyl has the highest value of 0.01 ± 0.001 followed by heptachlor (0.006 ± 0.002) then closely by dieldrin, aldrin with values of 0.005±0.002, 0.005±0.001, respectively. Endrin and Endosulfan II were not detected. Aldrin was reported in surface water in South Africa to range in value (µg/L) from 0.03-0.253, heptachlor (0.031-0.127), Dieldrin (<LOD-0.086). Dieldrin was detected in Ghanian soil to be within 0.02-0.03 mg/kg. All these values were higher than values obtained from this work for same pesticides but slightly above US MRL of 0.02 mg/kg for agricultural soils.[11,13] Other peaks shown were due to presence of untargeted pesticides. Four out of the eight OCPs; heptachlor, aldrin, O-Terpheny, dieldrin and were detected in soil samples A and B while heptachlor epoxide, mirex, endrin and endosulfan II were not detected in both samples. Concentration of all detected OCPs were lower than the United States maximum residue limit for agricultural soils.[14] Table 2 shows mean physicochemical properties of soil samples. pH, CEC, Ca, Mg, K, TEB, exchangeable acid and particle size distribution were all higher in soil sample B except clay particle (19.65 ± 0.27 %) that was more distributed in A. The pH of soil samples A (5.65±0.20) and B (6.19±0.20) were acidic. Low pH values were due to constant use of fertilizers on the site which conditions its acidity. pH values of samples A and B were low compared to that reported by Onuwa *et al.*[6] in analysis organophosphorus and OCPs in crops and soil on same site which ranged 7.2-7.3. Mean CEC (mol/kg), TEB (mol/kg), Ca, Mg, K, Na (mg/kg) for samples A and were (6.88±0.21, 5.88 ± 0.21, 2.82 ± 0.08, 2.60 ± 0.10, 0.25 ± 0.03, 0.21 ± 0.01) and (7.28±0.18, 6.23 ± 0.12, 2.92 ± 0.03, 2.77 ± 0.08, 0.28 ± 0.01, 0.25 ± 0.02), respectively (Table 2). CEC for samples A and B were all below 12 showing that the soil needs help through soil enrichment using a variety of amendments. The values were low compared to that of Onuwa *et al.*[6] whose CEC values ranged 7.8-8.1 mol/kg, indicating high content of sand. Soil CEC (below 12) indicates lack of significant nutrients. Soils with higher CEC values tend to hold more cations. Particle size shows that both soil samples were sandy-loam textural class which is the best for growing root crops. Table 3 shows coefficient of variation; a measure of precision for replicate data. Good precision is observed in all parameters except K for sample A (12 %).

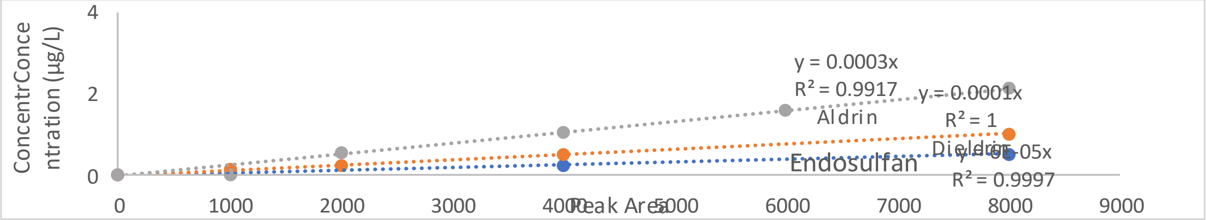

Figure 1: Calibration Curve for Aldrin, Dieldrin and Endosulfan Standards

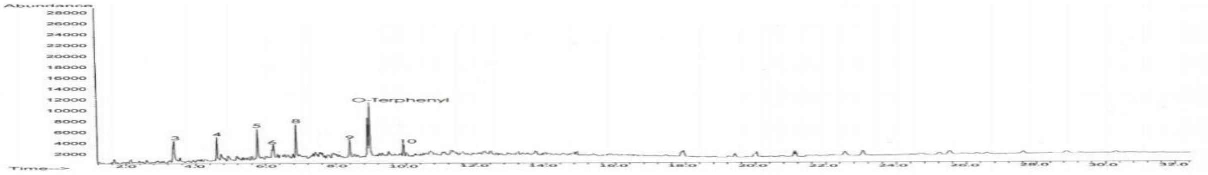

Figure 2: Representative Chromatograph of Organochlorine Pesticides for Sample B

Figure 1 shows the calibration curves for dieldrin, aldrin, endosulfan standards. Figures 2 shows representative chromatograph of OCPs residues for samples B.

4. CONCLUSION

This study detected four OCP residues in soil sample A and B while other four were not detected. This suggests their use by rice farmers in the area, which is tolerable for now but may accumulate to posts potential health risk of intended consumers of the produce and calls for proactive pesticide residue analysis of attendant crops.

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Impact Assessment of Refuse Dump Site on the Groundwater Quality of Orita-Aperin in Ibadan, Oyo state, Nigeria

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ABSTRACT

Ten groundwater samples were separately collected from each of eastern and western sides of Orita-Aperin abandoned refuse dump with the aim of determining the impact of Orita-Aperin refuse dump site on the groundwater quality. The water samples were analyzed for the following parameters using classical methods of analysis: pH, total alkalinity, total hardness, total solids, total suspended solids, total dissolved solids, chloride, sulphates and orthophosphates. The results of chemical analyses revealed that the level of total hardness (123.2 m/L ± 34.5); sulphate (1.917 mg/L ± 1.25) and chloride (99.6 mg/L ± 51.9) were higher in the groundwater samples at the western side than the level of total hardness (100.6 mg/L ± 44.15); sulphate (1.18 mg/L ± 0.72) and chloride (74.5 mg/L ± 38.86) at the eastern side of the dump. Although the level of these parameters at both sides fall below World Health Organization guideline values, but the pH of ground water at the eastern part (7.84 ± 0.24) was higher than the pH of water samples at western part (7.56 ± 0.48) although both values were within the range of 6.5-8.5 recommended as the pH of good drinking or potable water by World Health Organization. It becomes obvious that there is little or no contamination of groundwater in the area but routine analysis at interval from the area to assess any sudden increased in concentration of any parameter were hereby recommended.

KEYWORDS: Abandoned refuse dump site, groundwater quality, impact assessment, Orita-Aperin.

1. INTRODUCTION

Disposal of refuse is a priority problem within Ibadan metropolis due to poor management and illegal and wanton practice of dumping refuse at convenient locations. Many waste disposal sites are sources of environmental pollution because waste is still largely disposed without effective safety and control measures. Thus, groundwater environment is being assaulted with an ever-increasing number of soluble chemicals.[1] Groundwater is the water that occurs below the surface of the earth, where it occupies all or part of the void spaces in geological layers. It is also called sub-surface water, which flows overland and in rivers. Water is one of the most prizes resources of any nation and it occupies a prominent position among rural and urban dwellers. Commercial qualities of water come from surface and subsurface sources. Availability of surface water varies with season whereas groundwater can be obtained all the year round.[2] The volume of groundwater greatly exceeds that of all fresh water, lakes and reservoir combined which occurs in several geological formation aquifers at various depth. [2, 3]

The growth of man's agricultural, industrial and urban activities has caused the appearance of many environmental problems which share common disturbing characteristics. The increase of awareness of pollution and its effects has emphasized the importance of water quality management in maintaining our natural waters in a fit state for various purpose (e.g. for use as drinking water, for recreation or to assure the viability of the native biota.3 The quality of any water depends very much on its physical, chemical and biological characteristics. Water intended for human consumption must be free from organisms and from concentration of chemical substances that may be hazardous. Drinking water should be pleasant as circumstances permit, coldness, absence of turbidity, absence of colour and of any disagreeable taste or smell are utmost importances in drinking water supplies. Recreational water used for sport involving contact with the physical body of the sport men and women must be aesthetically acceptable, must not contain substances that are toxic upon ingestion or irritating to the skin and must be reasonably free from pathogenic organisms, colour, odour, hydrogen ion concentration (pH) and toxic metal ions.[4,7] The quality of water needed for industrial use depends grossly on the type of industrial process for which the water is needed. The quality for different industrial processes differs from one and another. Agricultural activities, mainly irrigation requires water that is reasonably free from pathogenic organism, pH and specific ion, so as to give high yield of product as different type of soil can retain different amount of water.

Thus, any substance that gives unfavourable alteration, undesired changes in the physical, chemical and biological character of the water is termed pollutant. According to (Sridhar and Ademoroti, 1984), it is said that "in some parts of Lagos, the most industrialized city in Nigeria, some of the waters showed highly acidic pH as low as 3.4.[5] In developing countries, most especially in Nigeria, there is an increasing deterioration of natural water, soils and air. A variety of wastes originating from domestic and industrial sources find their way into these systems due to lack of legislation and basic infrastructure such as sewage and hygienic disposal. It has been recognized that measures for groundwater protection should focus on the best possible safety provisions for future waste disposal sites. [6, 7] Thus, this research work investigated the impact of abandoned refuse dump site on the groundwater quality surrounding Orita Aperin, a residential area where people depend on wells for their domestic water supply in Ibadan, Oyo state and therefore evaluate the possible health consequences of selected water parameters by comparing the results obtained with those of drinking water standards set by statuary bodies such as World Health Organisation (W.H.O).

2. MATERIALS AND METHODS

2.1. Description of Study Area

The study area is Orita-Aperin, a residential quarter located within Ibadan Northeast Local Government Council Area of Ibadan City. The study area, Orita Aperin refuse dumpsite is located within longitudes $3°53^1$E and $30°56^1$E and latitude $7°21^1$N and $7°24^1$N. The area extent of the waste disposal site is about 0.26 by 0.22 km^2. It is owned and maintained by the Ibadan Solid Waste Management Authority. This site is bordered to the north and south by buildings, to the west by a mini-market and to the east by a banana plantation. The study area falls within the humid and sub-humid tropical climate of southwestern Nigeria with a mean annual rainfall of about 1230 mm and mean maximum temperature of 32°C

2.2. Water sample container.

Water samples of about 3 litres each were collected from the 20 wells into plastic bottles. In each case, the samples were collected with the rubber or plastic drawer. Samples were also properly labeled, noting the sampling points, time and date of collection. Plastic containers were used throughout the course of this work for both storage and sampling.

2.3. Prevention of sample contamination

Plastic containers which were the recommended sample containers were used throughout for both sampling and storage. Initially the containers were thoroughly cleaned and properly capped or corked after the collection of samples. Temperature measurement of the water samples were done in-situ on the field followed by determination of alkalinity which was done on the same day immediately the samples got to the laboratory.

2.4. pH measurement

The pH meter (Model 3150 (Jenway) which was used was first standardized in the buffer solution of 0.05 m pure potassium hydrogen phthalate of 4.0 pH value. The electrode was removed, rinsed and washed thoroughly with distilled water, blot dried and immersed in water samples AE1 - AE10 and AW1 – AW10 one after the other with successive rinsing with distilled water and blot drying after each sample.

2.5. Classical Methods of Analysis

Classical methods of analysis (i.e. gravimetry and titrimetry) was used for the determination of total alkalinity, total hardness, total solids, total suspended solids, chloride using silver nitrate solution method while instrumental methods were employed to analyse sulphate (using turbidimetric method (at 420nm) and Orthophosphate (by colorimetric method at 880nm). Calibration graph was prepared and distilled water (50 mL) was used as the blank to zero the instrument

3. RESULTS AND DISCUSSION

The results of the analyses of the samples collected were summarized in Table 1 and 2. For ease of identification, the samples collected were represented as sample AE1 to AE10 and AW1 to AW10 unless otherwise stated. The samples were collected from eastern and western sides of the dump site to determine the migration and direction of flow of the leachates from the dumpsite. The results were expressed in milligram per litre (mg/L). The results obtained from the two sides of the dump were compared and these were in turn compared to International standard like World Health Organization standard.

Table 1: Result of the groundwater analysis at the eastern side of Orita-Aperin abandoned refuse dump (concentration in mg/L (i.e. PPM)

Number/ parameters	pH	Total alkalinity	Total hardness	Total solids	Total suspended solids	Total dissolved solids	Chloride	Sulphate	Orthophosphate
AE1	8.00	90	58	286	72	214	44	0.95	0.0007
AE2	8.16	85	60	256	60	196	23	0.75	N. D
AE3	7.72	70	116	730	185	545	94	0.80	N. D
AE4	7.59	45	64	550	130	420	98	0.85	N. D
AE5	7.63	90	78	424	106	318	58	0.95	N. D
AE6	7.84	220	182	170	295	875	42	0.90	N. D
AE7	8.19	140	134	600	100	500	94	1.90	0.0001
AE8	8.14	190	166	622	155	467	164	3.10	0.008
AE9	7.60	65	90	544	136	408	82	0.60	N D
AE10	7.56	45	58	442	110	332	46	0.95	N. D
MEAN	7.84	104	100.06	562.4	134.9	427.5	74.5	1.18	0.0029
S. D	0.244	57.04	44.15	246.30	63.88	184.8	38.86	0.722	0.0036
MAX.	8.19	220	182	1170	295	875	164	3.10	0.008
MIN.	7.56	45	58	256	60	196	23	0.60	0.0001
W.H.O (mg/l)	65-8.5		500			1000	250	400	

Table 2: Result of the groundwater analysis at the western side of Orita-Aperin abandoned refuse dump (concentration in mg/L (i.e. PPM)

Number/ parameters	pH	Total alkalinity	Total hardness	Total solids	Total suspended solids	Total dissolved solids	Chloride	Sulphate	Orthophosphate
AW1	6.82	70	136	526	130	396	199	1.50	N. D
AW2	7.29	110	194	542	136	406	157	4.50	N. D
AW3	7.15	35	62	260	50	210	48	1.00	N. D
AW4	6.96	50	72	198	50	148	56	0.95	N. D
AW5	7.49	190	120	412	100	342	146	4.30	N. D
AW6	7.62	150	130	1284	400	884	138	2.00	0.0004
AW7	7.87	110	132	458	110	348	70	1.30	N. D
AW8	8.20	150	128	298	75	223	62	1.50	0.0043
AW9	8.11	150	138	436	200	200	68	1.55	0.00175
AW10	8.12	100	120	398	100	298	52	1.10	0.0009
MEAN	7.56	11.5	123.2	484.2	135.1	345.5	99.6	1.90	0.0018
S.D	0.48	470	34.5	287.0	97.8	198.0	51.9	1.25	0.0015
MAX.	8.20	190	194	1284	400	884	199	4.5	0.0043
IN.	6.82	35	62	198	50	148	48	0.95	0.0004
W.H.O (mg/L)	65-8.5		500			1000	250	400	

Table 3: Summary of the result of the groundwater analysis at the eastern and western sides of Orita-Aperin abandoned refuse dump (in mg/L).

No	Parameters	Mean ± eastern	S.D	Mean ± Western	S. D	W.H.O STD. (mg/l)
1.	pH	784	0.24	7.56	0.48	6.5 – 8.5
2.	Total Alkalinity	104	57.4	111.5	47.0	
3.	Total Hardness	100.6	44.51	123.2	34.5	500
4.	Total Solids	562.4	246.30	484.2	287.0	
5.	Total Suspended Solids	134.9	63.88	135.1	97.8	
6.	Total Dissolved Solids	427.5	184.8	345.5	198.0	1000
7.	Chloride	74.5	38.86	99.6	51.1	250
8.	Sulphate	1,18	0.72	1.97	1.25	400
9.	Orthophosphate	0.0029	0.0036	0.0018	0.0015	

KEY: W. H. O = World Health Organization Standard.**;** NO G. V. S = No Guideline Value Set.

4. CONCLUSION

The result of chemical analyses of groundwater samples from eastern and western sides of Orita-Aperin abandoned refuse dump revealed the impact of the dump on the groundwater quality most especially the chemical pollutants; some of which are above the international standard recommended by the World Health Organization. From Table 3 above, it can been seen that the level of total hardness (123.2 mg/L ± 34.5), Sulphate (1.97 mg/ L ± 1.25), and Chloride (99.6 mg/L ± 51.9) were higher in the groundwater samples at the western side than level of total hardness (100.6 mg/L ± 44.51), Sulphate (1.18 mg/L± 0.72) and Chloride (74.5 mg/L ± 38.86) at the eastern side of the dump (see Table 3) although the level of these parameters at both sides fall below World Health Organization guideline values. This result was in agreement with the result of (Ikem et al., 2002).[8] Equally, the pH of the groundwater at the eastern part (7.84 ± 0.24) was higher than the pH of water samples at western part (7.56) although both values were within the range of 6.5 – 8.5 recommended as the pH of good drinking or potable water by World Health Organization.[9] It becames obvious that there was little or no contamination of groundwater in the area at the time of this research but routine analysis at interval from the area to assess any sudden increased in concentration of any parameter were hereby recommended. The researcher, therefore appealed to Oyo state government to remove the dumpsite as a final solution to groundwater contamination and future waste disposal sites should be located far away from residential area.

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Science Teachers' Perceptions of the Systems Thinking Approach for Teaching and Learning Sustainability at Secondary Schools

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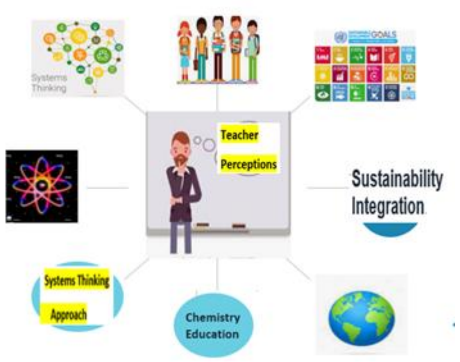
ABSTRACT

This paper presents the views of chemistry teachers about systems thinking through the concept of sustainability. The need for this study arose from the fragile nature of the climate, ecosystems and societies and how teachers as the drivers of new ideas for social, cultural, economic and financial development must play a role to maintain the earth's system through a more holistic approach to teaching. It has become important to identify topics in the syllabus that lend themselves to the practice of sustainability through systems thinking for the proper use and conservation of the earths' dwindling resources, mitigate overuse of carbon and its attendant effects, deal with climate change and reduce humanitarian challenges. Views of 40 in-service teachers were sought through a google survey to find out how teaching and learning of sustainability concepts could be reimagined to meet the needs of the earth system through systems thinking, and reinforce the teaching about complex systems across disciplines. Findings indicated that about 69% of in-service teachers perceived they had adequate knowledge about sustainability, but 75% had little to no knowledge about systems thinking. They admitted that there was the need for professional development courses in sustainability issues, and how they could be integrated into lessons using a systems thinking approach.



KEYWORDS: Chemistry teachers' Perceptions, Holistic approach, Professional development, Sustainability, Systems thinking.

1. INTRODUCTION

From research studies, sustainability principles and systems thinking are important to contemporary education and training.[1,2] Future teachers who apply systems thinking and sustainability approaches to life may enhance their potential to understand and solve some of the current and emerging global challenges as outlined by the United Nations Sustainable Goals and teach the same to their prospective learners.[3] Sustainability and systems thinking theoretical frameworks could be used as instructional models to facilitate desired change in science education.[2]

1.1. Sustainability

Sustainable development (SD) is development that meets the needs of the present without compromising the ability and needs of future generations.[4] Increased awareness of the importance of sustainable development, and the essential role that education could play, led to the development of the concept of Education for Sustainable Development (ESD). ESD is understood as an education that 'allows every human being to acquire the knowledge, skills, attitudes and values necessary to shape a sustainable future.' This means 'including key sustainable development issues into teaching and learning; for example, climate change, disaster risk reduction, biodiversity, poverty reduction, and sustainable consumption. Furthermore, the knowledge of content and suitable teaching strategies is required to infuse environmental sustainability (ES) into lessons so as to unleash the potentials of sustainability education.[3]

ESD is arguably at the heart of the 2030 Agenda for Sustainable Development and its 17 Sustainable Development Goals (SDGs).[5] The adoption of the 2030 Agenda for Sustainable Development has provided fresh impetus for Education for Sustainable Development. Under Goal 4, it is widely recognised that one of the most ambitious, interesting and challenging targets is Target 4.7, which aims to: “By 2030, ensure that all learners acquire the knowledge and skills needed to promote sustainable development, including, among others, through ESD and sustainable lifestyles, human rights, gender equality, promotion of a culture of peace and nonviolence, global citizenship and appreciation of cultural diversity and of culture’s contribution to sustainable development”. Achieving these goals requires a profound transformation in the way we think and act, [6] especially how classroom teaching and learning is enacted.

In order to implement ESD, it is important to review learning environments and encourage the whole-school approach. ESD calls for more focus on the integration of the pedagogies and thematic areas of ESD into training of teachers and trainers. It is interdisciplinary and transdisciplinary, meaning that no discipline can claim ESD as its own, but all disciplines can respond and contribute to ESD individually and/or collectively. New approaches that allow for holistic multidisciplinary teaching must be adopted, of which the systems thinking approach could be one.

1.2. Systems Thinking

Systems are combinations or groups of interrelated, interdependent or interacting components that form distinct collective entities. The outcome of the combinations, like a hybridised system functions better than if each were an individual component. A system could be non-material or physical. Systems thinking is therefore a set of synergistic analytic skills used to improve capability to identify and understand systems, predict their behaviours and devise modifications so as to produce desired effects. It is believed that these desired skills provide the ability to visualise, articulate, and solve uncomplicated and complex problems and concepts. This will enable the making of sensible decisions based on concrete available information.[7]

Systems thinking does not have a specific definition as slight modifications are possible depending on their different applications.[2,8] However, it can be viewed as a ‘holistic approach for examining complex’ real-world systems, in which the focus is not on the individual components of the system but on the dynamic interrelationships between the components and on the patterns and behaviours that emerge. Systems thinking has been applied in biology, economics, management, engineering, and the business world.[8,9]

Chemists can look at matter within the environment and not lose sight of its’ particulate nature.[9] The authors reiterate that systems thinking approaches should emphasise the interdependence of components of dynamic systems and their relationships with other systems, including cultural and environmental systems. Systems thinking has characteristics of problem-based and context-based learning. Using systems thinking concepts and tools together with context-based and problem-based learning approaches offers many opportunities to teachers. Furthermore, systems thinking approach would encourage problem-solving and knowledge integration that could span across disciplines.[7]

1.3. What is the possible role of science (chemistry) in the attainment of sustainability through systems thinking, within the sustainability development goals (SDGs) and ESD?

Chemistry is a central science that underpins basic aspects of a number of established and emerging sciences.[10] It must be acknowledged that chemistry has played a dual role in global development.[10] On a positive side, chemistry has provided knowledge and products such as energy, materials like polymers, plastics, semiconductors, pharmaceuticals, new vaccines as observed during the recent covid-19 pandemic, agrochemicals for crop protection and higher yields, and many more, so that it justifies chemistry’s claim to be the ‘quality of life’ science par excellence. On a negative side, the processes and products of chemistry, have paradoxically, contributed to a myriad of emerging global humanitarian problems, especially in less developed countries that do not have the needed resources to combat the emerging negative effects. The most obvious of these negative effects are the resultant global warming, rising sea levels, depletion of the ozone layer and increasing litter from non-decomposing plastics. In order to avoid multiple crisis, the world’s populace could be adequately educated on sustainability principles, and their benefits for now and posterity through a systems thinking approach.

Beyond the systems thinking approach, a conscious effort must be made to consider the integration of sustainability into school curricula, and also from the social sciences approach.[11] There is, however, little evidence of awareness of the SDGs and their importance among most practicing chemists, especially teachers, as a baseline study showed.[1] There is also little knowledge about education for sustainability development, as a study on Estwani teachers research shows.[12] The researchers found that creating awareness of environmental sustainability among learners could be an effective way of helping citizens to deal with sustainability challenges and so teachers had to be trained on how to teach with sustainability concerns in mind.

In chemistry lessons, it is difficult to visualise or imagine studies of ions, atoms and molecules without a study of compounds and the interactions of the named particles. Yet, chemistry plays a role in linking chemical, physical, biological, ecological and human systems towards sustainability. Current sustainability challenges would require the knowledge from chemical scientists and other collaborators from other disciplines. In order to enact this idea, chemists would necessarily have to be educated to engage in systems thinking and cross-disciplinary approaches.

Chemical activities could be seen as processes that could be in parts, whole or interactive. As put forth, chemistry components interact with many other systems in the environment which could have positive or otherwise effects.[9] Studying chemistry as a dynamic system would help to focus on the interconnected components that are coherently organised to advance knowledge, deliver useful applications and solve challenges, while reducing risks and improving safety and sustainability as intimated earlier. The many parts of chemistry also interact with many other systems, such as the environment and affect biological, ecological, societal, economic and other systems. This means that curriculum changes would have to be made in existing curricula to cater for the new realisations on how education for sustainable development in all sectors of formal learning, could impact the earth's systems positively.

Ordinarily, the teaching and learning of chemistry must be able to equip citizens with requisite knowledge to cope in a changing world, but is that the case? Does our knowledge of chemical principles make the world better or worse? Thus, how systems of knowledge in various disciplines relate to one another, to societal or cultural systems, and the entire world, becomes a very important issue to consider. A reductionist approach in science education and research is not sufficient to address global challenges such as sustainability, pollution, climate change, and poverty.[13] A more holistic approach (systems thinking) must be adopted so that boundaries around systems can be removed for the interconnectedness of systems to be apparent. Research that supports holistic, interdisciplinary, and transdisciplinary action must be considered.[11,1,14,10] In-service teachers (herein also referred to as teachers) must therefore be knowledgeable about these new ideas of teaching and implement them in their future teaching lessons.

In Ghana, some authors explored the possibility of embedding sustainability and humanitarian principles into chemical studies from a systems thinking approach.[15] Participants in that study acknowledged that the negative effect of chemistry and industrialization had translated into climate change, food crisis, financial crisis, poverty, water scarcity, poor health, war, injustice, migration and urbanization, and other humanitarian challenges and there was a need to work toward mitigating the identified challenges, because they were able to connect the systems as if there were no boundaries. The researchers gathered that a possible solution could be through the development of sustainability- and humanitarian-literate citizens through chemical education. Findings from questionnaires, interviews and inter-rated observation schedules indicated that it was feasible to embed sustainability and humanitarian principles from a systems thinking approach to inculcate into teachers the need to protect our ecosystem for posterity through chemical studies.

There was yet another study that also engaged teachers in introducing the concept of sustainability and systems thinking into lessons, but from an 'indigenous knowledge' approach. Indigenous science is fast becoming a pathway to teach the principles of sustainability and green science towards sustainable development, management and conservation of the world's ecosystems.[16,17] This study sought to explore participants' own ideas about sustainability and 'green' practices through the indigenous multi-stage production of palm-oil.

The production of palm oil was chosen as the object of study and trajectory for propagating issues of sustainability in science education, because in Ghana, palm trees from which palm oil is produced, abounds everywhere, apart from the Northern regions. It was therefore a common and familiar point to use in the indigenous science class to teach the principles of sustainability, its significance and green practices for preservation of natural resources from a systems thinking approach.

It is interesting to note that the Ghanaian science and chemistry syllabus for secondary schools do not categorically mention sustainable development, nor make references to systems thinking as one of its teaching approaches.[18,19] Neither do tertiary curricula mention systems thinking nor request that teacher trainers educate prospective teachers on education for sustainability development through it.[20] There are topics where sustainability issues through systems thinking could be incorporated. It is the singular role of the teacher to go through the subject content and identify topics with opportunities to adopt systems thinking approach to teach sustainability-inherent topics. Some common topics in official curricula for secondary and tertiary education in chemistry are:

S/N	Topic
1.	Particulate nature of matter
2.	Elements, mixtures and compounds
3.	Periodic property
4.	Stoichiometry
5.	Chemical reactions
6.	Acids, bases and salts
7.	Metals
8.	Organic chemistry
9.	Chemical bonding
10.	Industry and technology

A close study of the mentioned topics indicates that some of them easily lend themselves to and for the integration of sustainability principles and systems thinking in practical and contextual ways. Education for sustainability (ESD) through systems thinking does not require a new topic to be added to the curriculum but allows for an existing curriculum to be adapted to suit the needs of both the people and the environment.[14,15] Thus, a concerted effort must be made towards this revelation and institution

1.4. Gap

So far, the limited studies exploring teachers' knowledge of sustainability through systems thinking have focused mainly on university pre-service chemistry teachers and the design of integrated sustainability lessons using a systems thinking approach. A review of the literature revealed no studies or practices involving in-service teachers with more than a year of teaching experience. In this study, the term "practicing teachers" or "teachers" specifically refers to in-service teachers. This gap highlighted the need for the researchers to investigate the extent of in-service teachers' understanding of sustainability principles, systems thinking, and education for sustainability after sourcing for their perceptions.

2. RESEARCH QUESTIONS

1. What perceived knowledge do teachers have about sustainability?
2. What perceived knowledge do teachers have about systems thinking and its approach to teaching science?
3. What topics in the syllabus lend themselves to the teaching of sustainability principles and sustainable development from a systems thinking approach from teachers’ viewpoints?
4. What are teachers’ perceptions towards the integration of sustainability and systems thinking approach into their science lessons?

3. METHODOLOGY

In order to be able to answer the outlined questions in this case study, a mixed method approach was adopted where a 5-point Likert scale questionnaire to assess teachers’ knowledge on sustainable development (SD) and education for sustainable development (ESD) and semi-structured interviews were employed to gather data on perceived knowledge of sustainability concepts and the systems thinking approach. Parts of the questionnaire contained open-ended questions to elicit the teachers’ views on the relevance of sustainability to their teaching, whether they thought it was important for students to learn about sustainability, how they translated the ideas into their teaching, and if they required support with its integration from a systems thinking point of view. The interview items were built around the questionnaire items for the purposes of corroboration and the acquisition of in-depth views about the research topic, from participants. Eight participants were selected and interviewed. In qualitative research, smaller sample sizes can still provide rich, in-depth insights, with studies indicating that fewer than 20 interviews can often be enough to reach data saturation.[21] The decision to interview eight participants, representing 20% of the sample, is justifiable based on other factors. First, the study's specific goals and the homogeneity of the sample support the use of a smaller subset. Additionally, the quality of the interviews, along with the depth of the discussion, ensures that valuable insights can be obtained from a reduced number of participants.[22] Practical considerations, such as logistical constraints or time limitations, also contribute to the selection of a manageable sample size, making this approach both practical and effective.[23] In this case, interviewing 20% of the participants allows for a focused and meaningful exploration of the research question, while still being feasible within the study's constraints.

A case study allows for multiple forms of data collection to improve the findings' reliability and trustworthiness. For this study, the researchers chose to use questionnaires and interviews. Since the researchers engaged more than one teacher each from a different school, the type of case study used is an embedded, multiple-case study, which allows one to find a more in-depth understanding of a case and extend the generalisability of the findings. The use of mixed method was to help with the reliability and generalisability of the study’s findings.

Obtained data were analysed quantitatively and qualitatively. Combining both the in-depth, contextual views of qualitative data with the broader generalisations of quantitative approaches, mixed methods research can be used to produce a rigorous and credible source of data.[24] It also helps to build comprehensive understanding and enriches the evidence through different explored avenues. Furthermore, it does not only track intervention, if one is put in place, but provides opportunities for participants in a study to have a strong voice and share their experiences. Obtaining the quantitative findings and exploring them in-depth through qualitative means, is worth investigating.

The sample for the study was drawn from a population of science teachers in the Effutu Municipal Constituency in Winneba, Central region of Ghana. The study focused on 40 in-service science teachers who had taught for up to a maximum of ten years. A questionnaire on the length of continuity in science teaching, particularly chemistry topics, was used to screen the teachers and came up with a sample of eight science teachers, each from a different locality.

The philosophical position underpinning this study was that of the interpretivist worldview. An interpretivist paradigm is associated with the idea that individuals seek an understanding of the world they live in, and researchers rely fully on the participants’ perceptions of the situation being studied.[25] This philosophy, [26] is relevant to this study as teachers are social beings constantly interacting with students, fellow teachers, parents and community members.

Ethical considerations were carefully taken into account throughout the data collection process. Quantitative data gathered was subjected to descriptive statistical analysis to provide a clear overview of the responses. Qualitative data, obtained after data saturation, was organised into various themes and subjected to in-depth discussion. Issues of trustworthiness were ensured. In line with Creswell,[25] the strategies used to improve the trustworthiness of qualitative data were member checking, triangulation of data and external audits.[27] also recommended that experts in the field of research should review research instruments. In this study, the researchers asked subject experts and experienced educators to review and comment on the questionnaires and supporting interview schedules. The researchers created model answers to some of the questions in the questionnaire. Obtained data from the instruments used, were then analysed with respect to the requirements of the guiding research questions.

4. RESULTS

In order to ascertain whether teachers considered sustainability and the systems thinking approach relevant to the teaching of science (chemistry) in a holistic manner to maintain and save the earth, questions were directed and data collected to answer questions that would reveal the teachers’ perceived knowledge of the subject under study. For example, the questionnaire contained some closed items that teachers needed to show knowledge of, such as shown in Table 1.

Table 1: Examples of closed items

Item	Very familiar	Familiar	Somehow familiar	Not familiar	Not familiar at all
The concept of sustainability					
Rate your knowledge of sustainability					
How familiar are you with systems thinking approach?					

The items in Table 1 of the questionnaire were close-ended. Examples of the open-ended items were:

1. How did you hear about 'sustainability'? Explain
2. What is your idea about 'systems thinking'? Explain
3. Make a list of topics in the syllabus that you think can contain elements of sustainability
4. Which topics have you used to address sustainability issues holistically?

These responses required the participants to express themselves freely, and some of the mentioned ideas appeared more than once in other items.

The first research question (RQ 1) sought to find out about teachers' perceived knowledge about sustainability education. Data obtained is shown in Table 2.

Table 2: Responses on teachers' perceived knowledge about sustainability education

Categories	Very familiar	Familiar	Somehow familiar	Not familiar	Not familiar at all
Collated Responses (%)	43	26	31	0	0

The responses obtained indicated that more than half of the in-service teachers (67%) purported to have some knowledge about education for sustainable education.

Research question 2 sought to find out the perceived knowledge that teachers have about systems thinking and its approach to teaching science. Participants familiarity with the systems thinking approach was also assessed and the responses are presented in Table 3. This data was used to answer research question (RQ) 2.

Table 3: Responses on participants' knowledge about systems thinking

Categories	Very familiar	Familiar	Not sure	Not familiar	Not familiar at all
Collated responses (%)	0	5	20	25	50

The in-service teachers had practically no knowledge about the systems thinking approach to teaching science.

Research question 3 (RQ3) sought to find out topics in the science syllabus that teachers perceived lent themselves for the teaching of sustainability through a systems thinking approach. The topics that came up were nine in all. These were green economy, science and technology, energy, reproductive systems, farming systems and energy, earth science, industry and climate, waste management, and science and industry. After this provision, teachers' views on how often they integrated sustainability principles from systems thinking approach were sought, as shown in Table 4, to answer RQ 4.

Table 4: The integration of sustainability principles into lessons

Categories	I often do	I sometimes do	Not sure	I hardly do	Never do
Collated responses (%)	37	63	0	0	0

About 37% of participants responded that they often did, while 63% said that they sometimes did.

Table 5 gives an overview of teachers' perceptions towards the importance of the integration of sustainability into science curricula.

Table 5: Responses on perceptions towards the importance of integration of sustainability

Categories	Very important	Important	Not sure	Somewhat Important	Not important
Collated responses (%)	62	20	0	17	0

Table 5 shows that majority of the in-service teachers (62%) perceived the importance of the integration of sustainability education into existing curricula.

Table 6, which also answers RQ 4, gives an overview of teachers' perceptions of integration of systems thinking into science curriculum.

Table 6: Responses on the importance of the application of the systems thinking approach

Categories	Very important	Important	Not sure	Somewhat important	Not important
Collated responses (%)	53	10	20	17	0

In their responses to the importance of the application of the systems thinking approach, about half of the in-service teachers responded in the affirmative. Interestingly, from Table 6, only 63% indicated that the application of systems thinking was very important and important to their teaching of science

A further probe into what they thought about integrating sustainability principles and development into science lessons through open-ended questions and a focus group interview indicated that the teachers were not as prepared for the integration, due to little knowledge on it. They were divided on the question on how integrated sustainability lessons and education would contribute to their learners' awareness of global environmental issues and responsible citizenship. All the teachers agreed that it was important for students to be taught about sustainability issues holistically. Of eight teachers that were sampled for the interview, one did not explain why she thought it was important. These were some reasons given by the seven other teachers on the integration of sustainability principles:

- "*Students are our future leaders and persons of influence in the country"*
- *"To help make use of our natural resources without depleting them".*
- *"We are currently faced with the problem of warm weathers, rising sea levels and global warming which have resulted in change in weather patterns and humanitarian challenges. If students and everyone is aware, it would mitigate the challenges"*
- *"So that they can be part of the practices and implementation of sustainable development policies and strategies"*
- *"They are the present generation who will carry forward the message of sustainability practices in a more practical and meaningful way to the next generation"*

All of these reasons given by the teachers emphasised their understanding of the important role that learners must play in caring for the environment, passing the message of sustainability on and conserving resources so that future generations can also benefit from them.

When the teachers were asked about problems that could be anticipated with the integration of sustainability principles through the systems thinking approach they opened up to some possible challenges. Some of the issues that they came up with as current and anticipated barriers were:

- Lack of curriculum support
- Time constraints as per the current timetable
- Lack of resources to facilitate work within the constrained time
- Lack of support from administrators
- Lack of professional development opportunities

On the question of provision of support, the teachers unanimously agreed that they would be interested in receiving professional development in how to integrate sustainability principles and sustainable education through a systems thinking approach. They went on to enumerate some of their expectations during such training. They admitted that, to engage in ESD they would require training in:

- how to redesign their lessons
- the application of the Analysis, Design, development, implementation and evaluation (ADDIE) model or principle in their integration
- increased content knowledge about sustainability principles and educating about sustainability
- increased content knowledge on education for sustainable development

- systems thinking
- green economy
- climate change issues
- recycling of waste to prevent pollution
- conducting science activities on a small scale

The teachers mentioned that they needed resources to add meaning to their teaching of integrated or sustainability-embedded lessons holistically. They vaguely said that 'common resources' for teaching would have to be provided by their supervising authorities.

5. DISCUSSION AND SUMMARY

The results regarding teachers' familiarity with sustainability in education from a systems thinking approach showed varying degrees of understanding of the concept. When combining responses in the 'very familiar' and 'familiar' categories, a total of 69% of teachers demonstrate some level of familiarity with sustainability as observed from findings for RQ 1. This is a positive indication, suggesting that a good number of the teaching staff possesses at least a foundational understanding of the concept. However, the breakdown into the 'somehow familiar' category (31%) raises some concern on the level of familiarity. It would be beneficial to investigate the specific aspects of sustainability education that teachers find somewhat familiar, as this information could guide targeted efforts in professional development. Notably, there were no responses in the 'not familiar' or 'not familiar at all' categories, indicating that, among the surveyed group, there is no lack of awareness or knowledge about sustainability in education. These findings imply a solid foundation to build upon. Curriculum developers and professional development programmes can capitalise on this existing familiarity to deepen teachers' understanding and seamlessly integrate sustainability principles more comprehensively into educational practices to be in line with the United Nations Sustainable Development Goals and the Brundtland Convention targets.

It is noteworthy that there were no responses in the 'not sure' category, suggesting a level of conviction or clarity among respondents regarding the importance of sustainability. The breakdown into 'somewhat important' (17%) reflects a need to investigate the reasons behind this moderate level of importance to provide valuable insights into the specific factors influencing their views.

The absence of responses in the 'not important' category implies that none of the teachers outrightly dismissed the idea of integrating sustainability from a systems thinking approach into the science curriculum. This absence could be seen as a positive indicator for advocates of sustainability education.

To gain understanding of the varying degrees of importance, especially in the 'somewhat important' category, further exploration through follow-up interviews or surveys could provide qualitative data to inform strategies for curriculum development and teacher training.

The strong support for the importance of sustainability in the science curriculum aligns with broader educational goals related to sustainability and environmental literacy. These findings can serve as a basis for advocating policy changes or enhancing the curriculum.

In contrast to the teachers' response on sustainability, the study revealed a significant gap in in-service teachers' familiarity with the systems thinking approach in teaching science, with only 5% claiming to be 'familiar.' This suggests a potential gap in teacher training. Hence, targeted initiatives are needed to equip teachers with the knowledge and skills associated with systems thinking, as indicated by the high percentages in the 'not sure,' 'not familiar,' and 'not familiar at all' categories. These current observations are similar to other observations that have been made in earlier studies.[8,6]

From the findings for RQ 2, the noteworthy 50% of participants responding 'not familiar at all' suggests possible misconceptions or a complete lack of awareness regarding the benefits of systems thinking in teaching science. Given the low familiarity, there is an opportunity for professional development programmes to bridge the knowledge gap in applying systems thinking in the science curriculum. This aligns with the importance of ensuring that teachers have the necessary tools to implement contemporary pedagogical approaches.[15,7,2]

In response to RQ 2, the study indicates minimal participant knowledge about the systems thinking approach. This prompts further exploration into how introducing systems thinking could impact science education and teaching practices, opening avenues for pedagogical advancements as some researchers have also realized.[9]

The identification of nine diverse topics (from RQ 3) within the science curriculum that inherently contain sustainability-related issues, as purported by the teachers, such as green economy, energy, and waste management, emphasises the interdisciplinary nature of sustainability. This suggests that sustainability and systems thinking principles can be integrated across various science subjects. While 37% of participants claimed to often integrate sustainability principles into their lessons, the majority (63%) (See Table 4) said they did so only sometimes. This assertion also indicates a potential gap between teachers' self-perceptions and their actual integration practices, revealing a need for closer examination of their beliefs and necessary support for integration.

The participants provided insights into the perceived importance of integrating sustainability and the systems thinking approach into the science curriculum. The study's results (from RQ 4) regarding the perceived importance of integrating sustainability into the science curriculum among teachers show that a combined 82% of respondents, consisting of 62% selecting 'very important' and 20% choosing 'important,' express a positive view on the relevance of this integration. This indicates a clear recognition among teachers about the significance of incorporating sustainability principles into science education.

A substantial 53% of teachers regarded the integration of systems thinking as 'very important,' demonstrating a strong acknowledgment of its significance in science education. An additional 10% of respondents, recognising it as 'important,' further affirmed the positive view on incorporating systems thinking into the science curriculum. This support aligns with broader educational goals that emphasise critical thinking, interdisciplinary understanding, and holistic problem-solving. The findings suggest and confirm that systems thinking could be a valuable tool in achieving these educational objectives by in-service teachers on the field.[1,7] However, a portion of participants (20%) expressed uncertainty, and another group (17%) considered it 'somewhat important.' These responses among a minority of participants highlight the need to explore specific factors influencing their views. Further investigation through follow-up interviews or surveys could provide qualitative data to inform effective strategies for implementing systems thinking in science education.

The absence of responses in the 'not important' category signifies a collective agreement on the value of integrating systems thinking into the science curriculum. This positive reception aligns with the potential benefits associated with a systems thinking approach as observed by York et al.[8] This creates a supportive environment for curriculum designers, indicating that there is an endorsement for developing and implementing curriculum changes that incorporate the principles of systems thinking into science education.

The study reveals several implications based on teachers' perspectives on integrating sustainability and systems thinking principles into the science curriculum. Although the teachers agreed that sustainability issues were relevant to science and that it was important for learners to be taught about sustainability issues, the findings show that the teachers had uncertainties about ESD during the discussion interview session. This meant that their foundations for developing integrated lessons with environmental sustainability were not solid. These uncertainties could affect how they plan and conduct lessons. According to Burmeister and Eilks,[3] a positive attitude, knowledge of content, and suitable teaching strategies are requirements for infusing SD into teaching.

The open-ended questions and focus group interview exposed a discrepancy between teachers' self-reported preparedness, as indicated in closed-ended questionnaires, and their actual readiness. There was a clear mismatch of what they wrote about sustainability and the enacted, in their oral submissions. The teachers expressed varied opinions on the impact of integrated sustainability and systems thinking lessons on learners' awareness of global environmental issues and responsible citizenship.

Some oral responses that contradicted what they had indicated in the questionnaire are presented:

- *I am not sure about all this sustainability and more, the systems thinking, but could be good.*
- *We teach 'our world and our people' which looks at world issues and culture, do we need sustainability again? I mean specifically? Maybe, it should be on the timetable for emphasis.*
- *I wonder about integrating these principles. What about work load for teachers and learners?*
- *This is part of the practices and implementation of sustainable development policies, but not enshrined clearly so you wonder where to fix it sometimes. Hmmm. So much work.*

Despite differing views on the integration of these principles, all teachers unanimously agreed, even if implicitly, on the importance of teaching learners about sustainability issues. The reasons that they provided highlight a shared understanding of the critical role that learners play in environmental stewardship and the transmission of sustainability practices holistically to future generations. The teachers acknowledged challenges in their oral submissions about the incorporation of sustainability and systems thinking into their lessons, including lack of curriculum support, time constraints, resource limitations, lack of administrative support, and insufficient professional development opportunities. These challenges reflect practical barriers that need to be addressed for effective integration. According to researchers such as Jackson and Hurst,[2] York et al.[8] and Mahaffy et al.[9] effective integration processes must be put in place to attain the United Nations goals on ESD. The proposition by Hanson and Hanson[15] could be a valuable asset, if institutions could adopt or adapt the guiding principles that have been suggested for the integration of sustainability principles through the systems thinking approach.

The unanimous interest among teachers in receiving professional development on integrating sustainability principles through a systems thinking approach signigies their recognition of the need for training. The specific expectations listed, such as redesigning lessons and enhancing content knowledge, highlight the multifaceted nature of the skills they seek. While teachers expressed a need for additional resources to enhance their teaching of sustainability-embedded lessons, there was a lack of specificity regarding the required resources. The general call for ‘common resources’ suggests a desire for support from supervising authorities in providing the necessary tools for effective integration as found by Hanson[11].

The findings of teachers’ pereptions, emphasise the complexity of views on integrating sustainability and systems thinking into science education, highlighting the need for professional development, resources, and a systemic approach. The study underscores the importance of aligning teachers’ perceptions with their actual practices and addressing practical challenges to ensure effective integration of sustainability and systems thinking principles in the science curriculum.

5.1. Interpretive reflections

It is becoming clear that holistic teaching from the systems thinking approach should go beyond a focus on knowledge and skills to promote values and attitudes for sustainable development and empower responsible citizens to take action for change. Institutions should see themselves as experiential places of learning for sustainable development, and should therefore orient all their processes towards principles of sustainability from a systems thinking approach.

6. CONCLUSION

The study sought to find out teachers' perspectives on integrating sustainability and systems thinking principles into high school science curricula. The open-ended questions and focus group interview exposed a discrepancy between teachers’ self-reported preparedness which was high, and their actual readiness which was low. Findings indicated that teachers perceived that they were familiar with knowledge about sustainability but not familiar with knowledge about systems thinking and its approach. Findings revealed that they were aware of topics that lent themselves to the teaching of sustainability principles. However, they intimated that they would require training in the integration of sustainability and the systems thinking approach into existing lessons. It could be deciphered from findings that an experienced teacher is not necessarily an expert teacher in emerging concepts such as the idea of and integration of the systems thinking approach in teaching practices as majority of them did not truly understand how they would integrate the new ideas into their existing lessons. Emerging concepts must be taught to practising teachers through professional development sessions to ensure that educational transformations are effective.

7. RECOMMENDATION

An important recommendation would be to organise professional development courses for teachers on new ideas that must be integrated into their lessons. Qualitative approaches are recommended to identify and understand emergent ideas and new answers provided by learners; an aspect that is crucial to the dynamic and transformative aspects of ESD. Another recommendation would be for policy changes within schools and Departments of Science Teacher Education to support teacher development. For a start, it would be helpful if samples of integrated lessons that have sustainability issues embedded into them are made available for use by teachers, while they learn how to integrate the necessary sustainability principles into topics that would lend themselves to such from a systems thinking approach.

8. ACKNOWLEDGEMENTS

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Antimicrobial Screening of the Ethanol Extract and Alkaloid Fraction from the Root of *Acalypha hispida*

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ABSTRACT

Acalypha hispida is a fast-growing tropical shrub belonging to the family *Euphorbiaceae*. The plant is used in traditional medicine to treat various ailments including leprosy, skin rashes, ulcers, wounds, diarrhoea, and gonorrhoea. This study was aimed at screening of *Acalypha hispida* plant for its antibacterial activities. The cytotoxicity was carried out using a brine shrimp lethality test (BSLT) assay. Antimicrobial activity of the ethanol root extract and an alkaloid fraction of *Acalypha hispida* against clinical isolated species of Staphylococcus *species, gonorrhoea, Bacillus anthracis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Aspergillus actinomycete,* and *Candida albicans* were investigated using micro dilution agar method MIC and MBC methods. The result obtained from the BSLT gave LC_{50} of 31.41 µg/L for alkaloid fraction and LC_{50} of 62.28 µg/L for ethanol extracts indicating higher toxicity of alkaloid fraction to brine shrimp larva. The alkaloid fraction showed high antimicrobial activity against all the microorganisms tested indicating a broad spectrum of activity. The result from the ***in-vitro*** inhibitory activity revealed that the alkaloid fraction was more active against the Gram-negative bacteria (*E. coli* and *K. pneumonia*) with inhibition zones of 17 -33 mm. The high antimicrobial activity observed in the alkaloid may be due to the presence of some phytochemicals with high antimicrobial activity.

KEYWORDS: Medicinal plant, Microorganisms, Alkaloids, Cytotoxicity, Phytochemicals

1. INTRODUCTION

Medicinal plants contain substances that are of great importance to the health of individuals and communities.[1] They play a significant role in providing primary health care services to rural people. They also serve as therapeutic agents and important raw materials for the preparation of traditional therapies and some modern drugs.[1] Plants are rich sources of secondary metabolites, such as alkaloids and saponins which have been found in *in-vitro* studies of *A. hispida* to possess antimicrobial, anti-inflammatory, and anti-diabetic properties[2]. These metabolites are low molecular weight substances that are essential for producing constituents that are inhibitors of other organisms which compete for food supply or regulators of the cellular differentiation process[3]. Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. The name alkaloid is derived from the word alkaline and is used to describe any nitrogen-containing compound. [4]Alkaloids are heterocyclic nitrogen compounds that are reported to be useful in treatment of numerous diseases including Human Immunodeficiency Virus (HIV) Infection, trypanosomiasis,_malaria among others[5]. They are also reported to have antimicrobial and anti-diarrhoea activity due to their effect on transit time in the small intestine and their ability to interact with microbial deoxyribonucleic acid (DNA) [6,7.] The increase in and over use of antibiotics in the treatment of microbial infections is preserving bacterial resistance against available antibiotics.[8] Thus, newer more efficacious antibiotics are needed to treat microbial infections. Approximately 20% of the world's plants have been subjected to pharmacological or biological tests as natural products of plant origin are important sources of constituents that could be developed into drugs, dyes, fragrances, and pesticides.[9] *Acalypha hispida*, (Chenille plant) is a flowering shrub belonging to the family Euphorbiaceae, subfamily *Acalyphinae*, and genus *Acalypha* is the fourth largest genus of the Euphorbiaceae family. The plant is also known as the "Philippines Medusa", "red hot cat's tail" and "fox tail'' in English. The root and flower decoction are used for kidney ailments and as a diuretic. Leaf poultice is used as a cure for leprosy, the decoction of leaves and the flowers are taken internally as laxative and for treatment of gonorrhoea. Bark is used as an expectorant for asthma[10]. Phytochemicals component in the plant show the presence of alkaloids, anthraquinones, saponins, phenolics, steroids, coumarins, and glycosides which exhibit antifungal, anti-ulcer, and antitumor properties have been previously investigated. The plant contains ellantogitannis such as acylpyridines M_1, M_2 and D_1, anthocyanins namely, cyanidin 1-O-(2''-galloyl-β-galactopyranoside), cyanidin 3-O-(2''-galloy-β-galactopyranoside) and cyanidin3-O-β-galactopyranoside. [11, 21]. In the present study, we report the antimicrobial activities of the ethanol and the alkaloid extract of the root of *Acalypha hispida* grown in northern part of Nigeria.

2. MATERIALS AND METHODS

2.1. Plant collection

Fresh roots of *A. Hispida* were obtained from the biological Garden of Ahmadu Bello University, Zaria, Kaduna state, Nigeria. August 2023. The identity of the plant was authenticated in the herbarium section of the biological Sciences Department Ahmadu Bello University Zaria by comparing it with the herbarium Voucher specimen and voucher number ABU02643 was obtained for future reference.

2.2. Sample Preparation

The root samples were carefully air-dried under a shed in the laboratory at room temperature. Pulverized into uniform powder using a wood mill machine and the powder was kept in a jar at room temperature.

2.3. Extraction of Plant Material.

The powdered plant materials were extracted using absolute ethanol. About 100 g of the pulverized root sample was soaked in 500 ml of absolute ethanol for 72 hours using maceration method of extraction and the mixture was filtered using two folds of whatt man No. 1 filter paper. The extract was concentrated at 40°C using a rotary evaporator.

2.4. Phytochemical Analysis

The ethanol extract was subjected to preliminary phytochemical tests using standard methods as described. [12, 13]

2.5. Alkaloid Extraction

Alkaloids were extracted following the method reported by [7] the dried powder of the plant root (50 g) was mixed with 20% $^{v}/v$ ethanol with distilled water and dried at room temperature before the extraction of total alkaloids. The granulated powder was extracted with benzene for 6h. The extract was shaken with three successive portions of 25 mL of 5% sulphuric acid and decolorized by heating with activated charcoal. The hot solution was then filtered using Whatman No.1 filter paper. The filtrate was percolated and the solvent was evaporated under vacuum. The residue was then dissolved in water/hydrochloric acid mixture at pH 2.5, and allowed to seep. The collected solution was adjusted to pH 8 with ammonia and washed (6 x 150ml) of dichloromethane. Then, the dichloromethane was evaporated and the resulting residue was concentrated to dryness under reduced pressure to yield the solid alkaloid extract which was confirmed using Dragendorff reagent to give purple colour.[15]

2.6. Brine shrimp lethality test

Brine shrimp toxicity test: A portion of 70 g of Brine shrimp (*Artemia salina*) eggs was added to 250 ml ^{of} seawater in a beaker and kept for 48 hours for the eggs to hatch into shrimp larvae. A portion of 0.2g of each extract dissolved in 2ml of its various solvents of extraction. 50, 5, and 1μ/L of each solution were drawn into vials and allowed to evaporate within 24 hr. Two drops of DMSO were added and made up to 2ml with distilled water corresponding to concentrations of 1000, 100, and 10 μg/ ml respectively. Each dosage was prepared in triplicates including the control. Ten (10) shrimp larvae were added to each vial. The number of the surviving shrimp at each dosage and the control was recorded after 24hrs and the $LC_{50,}$ was computed using Finney probit analysis Software. In this assay, a drop of dimethyl sulphoxide (DMSO) was added to test and control vials to enhance the solubility of the test materials. All tests and analysis were carried out in triplicates and the results obtained were averaged.

2.7. Antimicrobial Assay

The diluted concentrations range of 0.5-5.0 x10^2µg/cm^3 of extracts was prepared and were tested for their antimicrobial properties using the agar-well technique.[15] The assay for antimicrobial activity was carried out using standard clinical isolate of *Staphylococcus aureus, Lactobacillus species, Staphylococcus faecalis, Bacillus subtills, Bacillus anthracis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumonia,* and *Staphylococcus diderm* and fungal isolates used included A*spergillus funigatus, Aspergillus actinomycete,* and *Candida albicans*. The antimicrobial susceptibility test was conducted using the method earlier described by [15]. The tests were carried out using a stock concentration of 100 mg/ml prepared by dissolving 1 g of the crude extract into 10 ml of sterile distilled water. The dilution ratio for Gram-positive bacteria and Gram-negative bacteria was 1:1000 and 1:5000 respectively using peptone water (15). About 0.5 ml of the dilute cultures were aseptically inoculated on the surface of sterile Petri dishes containing sterile solid nutrient agar. Discs impregnated with the crude extract at the concentration of 5 mg/disc were aseptically mounted on agar and thereafter incubated at 37^{o}c for 24 hours, the inhibition zone was observed and then recorded in millimetres using a transparent meter rule. The standard drugs used are penicillin and ciprofloxacin respectively.

2.7.1. Test of Minimum Inhibitory Concentration (MIC)/Minimum Bactericidal Concentration (MBC)

The MIC and MBC of the extract were determined by the micro dilution agar method. About 2-fold serial dilution of the extracts prepared in sterile distilled water to achieve a decreasing concentration ranging from 160 to 1.25 mg/ml in 9 sterile tubes labelled 1 to 9. A sterile cork borer of 8 mm diameter was used to bore well in the resolidified Mueller Hinton agar (MHA) plates and 100 µL of each dilution was added aseptically to the wells in triplicate that had microbe isolate seeded with the standardized inoculums (1.5 X 10^8 CFU/ml). 100 µ methanol was introduced into the well-in-place extract as the control. All the test plates were incubated at 37 ^{0}C and were observed for growth after 24 hours. The lowest concentration of an extract showing a clear zone of inhibition was considered as the MIC. In the determination of MBC, a 100µl aliquot from the tube showing MIC was placed on an MHA plate and spread over the plate. After incubation at 37 ^{0}C for 24 hours, the plates were examined for the growth of a bacterium to determine the concentration of the extract at which 99.9% killing of bacterial isolates was achieved.

3. RESULTS AND DISCUSSION

3.1. Results

The results from the studies are presented in Tables 1 to 4.

The result obtained from the brine shrimp lethality test BSLT (Table 1) gave LC_{50} of 61.4 µg/L for alkaloid fraction and LC_{50} > 100µg/L for ethanol extracts respectively.

Table 1: Brine–Shrimp lethality test result of *Acalypha hispida stem bark,*

Plant Extracts	LC_{50} µg/ml
Ethanol Extract (EE)	62.28
Alkaloid Fraction (AK)	31.14

The results of the phytochemical screening of the ethanol and alkaloid extracts showed the presence of different secondary metabolites constituents (Table 2) present in the plant.

Table 2: Phytochemical Constituents of the Ethanol Extract of *Acalypha hispida* Root

Phytocompounds	Ethanolic extract
Alkaloids	+
Anthraquinones	+
Carbohydrates	-
Saponins	+
Phenolics	+
Tannins	+
Phlobatannins	+
Terpenoids	+
Steroids	+
Flavonoids	+
Glycosides	+
Coumarin	+

Key: + = present, - = absent

The result of the antimicrobial activity presented in Table 3, revealed that the alkaloid fraction was active more against the Gram-negative bacteria (*S. aureus, E. coli* and *K. pneumonia*) with inhibition zones of 17-33 mm, while the ethanol extract was active against all other test microorganisms, indicating a broad spectrum of activity (Table 3).

Table 3: Zone Inhibition of ethanol and alkaloid extract of *Acalypha hispida* Root against microorganism

Microorganism	Conc. x10^2µg/cm^3	EE	AK	PEN	CP	*C
Staphylococcus aureus	0.5	30	28	N	25	N
Lactobacillus spp	1.0	14	15	N	23	N
Staphylococcus faecalis	1.5	33	23	N	18	N
Gonorrhoea	2.0	23	19	N	21	N
Bacillus anthracis	2.5	19	N	20	31	N
Escherichia coli	3.0	12	33	24	N	N
pseudomonas aeruginosa	3.5	14	12	26	33	N
salmonella typhi	4.0	24	21	23	N	N
Klebsiella pneumonia	4.5	25	33	25	31	N
Staphylococcus epiderm	5.0	21	17	19	21	20

N= No Inhibition *C= Negative control EE= Ethanol extract, AK=Alkaloid extract, PEN= Penicillin, CP= Ciprofloxacin.

The result of the antifungal activity presented in Table 4, revealed that the ethanolic extract was active against the tested fungi stain with inhibition zones of 15-33 mm, while the alkaloid fraction gave no inhibition against all tested fungi strain (Table 4).

Table 4. Zone Inhibition (mm) against fungal isolates using Ethanol and Alkaloid *Acalypha hispida* Root extracts

	Zone of inhibition (mm)		
Microorganism	EE	AK	x10^2µg/cm^3
Aspergillus funigatus	32	N	6
Aspergillus actinomycete	32	N	6
Candida albicans	33	N	6

N= No Inhibition EE= Ethanol extract, AK=Alkaloid extracts

3.2. Discussion

The result obtained from the brine shrimp lethality test BSLT gave LC_{50} of 61.4 µg/L for alkaloid fraction and LC_{50}> 100µg/L for ethanol extracts indicating high toxicity of alkaloid fraction to brine shrimp larva. The results of the phytochemical screening of the ethanol and alkaloid extracts showed the presence of different active constituents like flavonoids, terpenoids, tannins, phlobatannins, alkaloids, saponins, phenolics, anthraquinones, steroids, glycosides and coumarins (Table 2). These Phyto-compounds have been reported to be responsible for the antimicrobial properties displayed by many medicinal plants[17]. The result from the *in- vitro* inhibitory activity revealed that the alkaloid fraction was active more against the Gram-negative bacteria (*E. coli* and *K. pneumonia*) with inhibition zones of 17-33 mm, while the ethanol extract was active against all other test microorganisms, indicating a broad spectrum of activity (Table 3). The results of the effect of the extracts on the fungal isolates are shown in table 4. The ethanol extract inhibited all the tested fungal isolates with inhibition of (32-33mm), while the alkaloid had no effect on the tested microbes (see Table 4). Thus, their presence in this plant may be responsible for the remarkable antimicrobial effect observed. The results of the *in- vitro* inhibitory activity of the alkaloid fraction show that the ethanol extract are quite appreciable when compared to the mean inhibition zones produced by the control antibiotics and the fact that the standard antibiotics are in the purified and concentrated form whereas the extracts are crude and may harbour both pharmacologically and non-pharmacologically active compounds (see Table 2-4). In the test of Minimum Inhibitory Concentration (MIC)/Minimum Bactericidal Concentration (MBC), the plates were examined for the growth of a bacterium to determine the concentration of the extract at which 99.9% killing of bacterial isolates was achieved.

The high activity exhibited by ethanol extract against tested strains of microbes shows that the root extract could be used as an alternative medicine in treating *staphylococcal* infections pending when the active ingredients of this plant would be isolated, chemically identified, and purified for further evaluation. Alkaloids generally have been noted for their ant malarial and antibacterial activities although it seems their mechanism of action on microbes remains unclear.[7, 17] The high activity observed more on the Gram-negative bacteria by the alkaloid extract is noteworthy and could have been a result of a possible interaction between these alkaloids and some constituents of the Gram-negative cell wall thereby causing cytotoxic damage to this group of bacteria. This suggestion is on the basis that structurally, Gram-positive and Gram-negative bacteria differ only in their cell wall composition.[18] Therefore, the alkaloid extract could be explored as a narrow-spectrum herbal drug especially in cases of gastrointestinal infections since the tested bacteria strains are clinical isolates. The inhibitory mechanism of the phytochemicals extracted with ethanol on the radial growth of the filamentous fungi could be that of blocking the synthesis is of the cell wall constituents or possible interference with the replication of genetic material to prevent cell division since it was recorded that the Molds did not grow at all.[19]

4. CONCLUSION

The maximum antimicrobial activities exhibited by the ethanol root extract of *A. hispida* against bacteria (Gram-positive and negative) and fungi respectively suggest that the extract may be used for the treatment of typhoid, malaria, boil, respiratory tract, and other diseases caused by the test organisms. The alkaloid fraction significantly inhibited the growth of Gram-negative bacteria, and thus could serve as a good first-line drug for infections caused by Gram-negative bacteria.

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Enhancing Food Security in Nigeria Using Climate –Smart, Green Chemistry Technology for Stabilization of Niger Delta Coastal Soils: Bayelsa Experience

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ABSTRACT

The Nigerian oil reserve is largely found in the Niger Delta Region, (NDR) where most of the oil bearing communities are found along the coastal line. Majority of the land areas in these coastal communities are characterised by very poor soil chemistry. Bayelsa State, being at the center of coastal lines, is denoted as swampy area with soils characterised as strongly acidic and strongly clayey in most places. Being swampy, sand-filling is a common trend. It is, therefore, not a profitable venture to grow economically viable agricultural raw materials that could promote food security. Consequently, most of the economically viable raw products are imported into Bayelsa from other regions, resulting in high cost of living, hunger and poverty. The NDR is reputed for petroleum – based economy and this in turn is pivotal to national economy. This study explored the option of transforming these unsuitable land areas into arable land by modifying soil chemistry via an innovative in-country climate – smart, green chemistry technology. Results from pilot study showed that the applied technology positively modified soil chemistry and enabled the production of economically viable agricultural raw products with potential for transformation to value-added products. Study is a contribution to using chemistry as a tool to enhancing food security creating potential platform for national economic diversification, capable of job creation, reduction of hunger and poverty, in line with sustainable development goals.

KEYWORDS: Green chemistry, food security, soil stabilization, Climate-smart.

1. INTRODUCTION

Nigeria is presently facing food security crisis, attributed to insurgencies, conflicts and insecurity that have disrupted food production value chain resulting in food shortages. Aside these disruptions, there are other factors such as poor infrastructure. economic challenges, poor agricultural practices, climate change and poor soil chemistry with the coastal communities more affected. The Niger Delta region of Nigeria, including Bayelsa State, is characterised by a complex network of rivers, mangroves, swamps, coastal areas, soil degradation, erosion, flooding, and impact of oil spills that have significantly reduced the quality of arable land in the area, limiting the region's food production potential via agricultural productivity and threatening food security[1,2].

Green chemistry offers a promising approach for addressing these challenges through the development and application of environmentally friendly technologies that reduce or eliminate the use and generation of hazardous substances[3]. Climate-smart, green chemistry technology aims to stabilise and remediate coastal soils by enhancing soil structure and chemistry, achieved through sustainable soil regeneration and management practices including the use of climate – smart commodities, which can enhance soil carbon sequestration and improve the resilience of the soils, enhancing food security, contributing to sustainable development goals by promoting environmentally responsible crop production practices and mitigating the effects of climate change[4,5,6,7]. Addressing soil chemistry issues is very critical to proffering solution for improved crop production and food security in Bayelsa coastal communities and similar coasts in Nigeria, in general.

The objective of this study was, therefore, to explore the option of transforming these unsuitable land areas into arable land by modifying soil chemistry via an innovative climate-smart, in-country green chemistry technology. In this study, various food crops served as bio - indicators to assess the potency of the innovative technology in soil regeneration. However, this paper will focus only on one crop - Scotch Bonnet peppers (*Capsicum Chinense*).

2. MATERIALS AND METHODS

2.1. Soil Stabilization Procedure

This pilot study was conducted in Federal University Otuoke, Bayelsa, Nigeria using twelve plots of virgin mangrove swampy land area allocated to the research group (AgriChemTech) by the University Management in 2021. The area was cleared and delineated into different plots of dimension 30 m by 12 m each. Each plot was divided into two columns with each column further sub-divided into 12 to 13 units. Each unit was referred to as a windrow and was regenerated using customised, climate-smart commodities [plant-based polymers (EcoGrow and EcoFix)], with the basic feedstock preparation reported in[8] but modified by the use of biopolymers produced from invasive plant species found in the marine waters of Otuoke. Regenerated and stabilized windrows were then allowed to equilibrate for one week before use. Soil samples were collected before and seven days after stabilization procedure and analysed for relevant properties following the method of [9] but only pH and electrical conductivity (EC) are reported in this paper. Crop growth dynamics were then monitored with time.

2.2. Extraction of Humic and Fulvic Acids from EcoGrow and EcoFix with Subsequent Chemical Characterizations

Extraction of humic acid (HA) and fulvic acid (FA) and subsequent chemical characterisations were carried out, adopting procedures reported in [10]. The extracted and purified HA and FA were subjected to spectroscopic analysis using an FTIR spectrophotometer at the Multidisciplinary Central Research Laboratory (MCRL). University of Ibadan. Optical density (E_4/E_6 ratio) was evaluated using UV-Visible spectrophotometer (HACH DR390 Model) with absorbance values taken at 465 nm and 665 nm for E_4 and E_6 values respectively.

2.3. Assessing the Potency of the Regenerated Soil for Crop Growth Enhancement

Bio-indicator crops including but not limited to plum and cherry tomatoes, bell peppers (yellow and red variants), scotch bonnet peppers (yellow, orange and red variants), water leaf, New Zealand spinach, fluted pumpkin, exotic grape vines (green, red and purple variants), okro, water melon, eggplant (green and white variants), broccoli, cucumber, plantain, carrot, cassava and cabbage. Soil nutrient management and crop protection were achieved by the application of a tailored plant-based biopesticide (EcoShield). All the three products (EcoGrow, EcoFix and EcoShield) were prepared by our multidisciplinary research group (AgriChemTech, Federal University Otuoke). The humic fractions (Humic acid and Fulvic acid) of EcoGrow and EcoFix were characterised via infrared spectroscopic, volumetric and UV-visible spectrophotometric analyses for functional group evaluations following the procedures of [8]. Watering of indicator crops was carried out three times a week using irrigation system designed by the research group. Irrigation water was treated using methods described in [11]. It is good to emphasize that this study neither involved genetic modification, use of chemical fertilizer nor chemical pesticide but involved 100% green crop production and pest management. Screen house was erected over each plot to provide a controlled environment only. Similar crops grown without the application of the technology under investigation served as controls.

3. RESULTS AND DISCUSSION

3.1. Information from Acidity Group Contents, Infrared and UV-Visible Absorptions of Extracted Humic and Fulvic Acids

(a) Infrared absorptions: Information from infrared absorption characteristics (Table 1) showed that the molecular fragment of the humic acid (HA) and Fulvic acid (FA) were enriched with chemical bonds such as O-H, indicating hydroxyl groups for aliphatic hydroxyl (ROH) and phenyl hydroxyl (PhOH) found in carboxylic acids (CO_2H), alcohols and or phenols. N-H bond indicated the presence of nitrogenous compounds such as amine (NH_2) and amides. The C=O bond could be attributed to the presence of carboxylic acid, aldehyde (RCHO), ketone (RCOR’) and amide (RCONR’). Data also suggested the presence of unsaturated hydrocarbons (alkene and aromatics) and substituted hydrocarbons, which could be cyclic/acyclic.

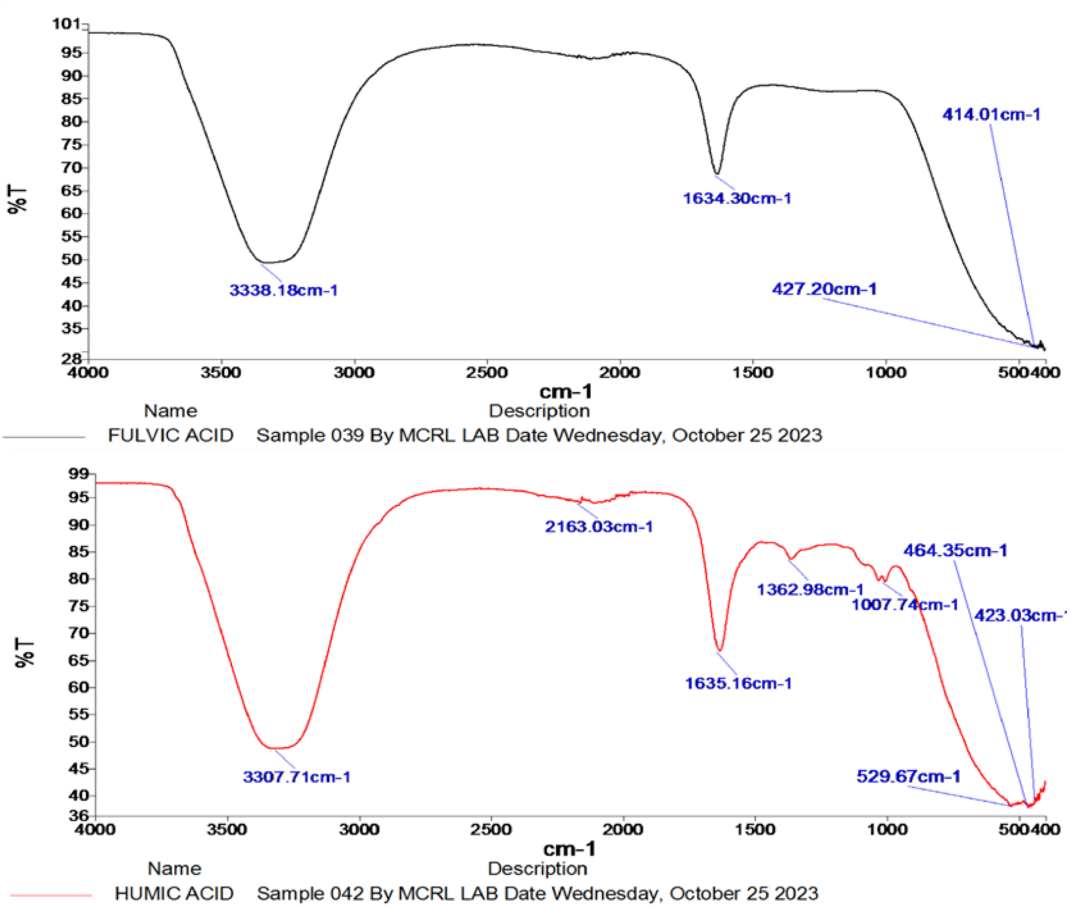


Fig.1: Infrared absorption spectra for humic acid and fulvic acid extracted from soil health enhancing biopolymer used in this study

(b) Acidity Group Content: Carboxylic acidity measures the concentration of carboxyl groups (-COOH), phenolic acidity measures the concentration of phenolic hydroxyl groups (-OH) and total acidity measures the total concentration of both, expressed in units of meq/g.

Table 1: Infra-red Absorption Characteristics of Humic and Fulvic Acids Extracted from EcoGrow and EcoFix

S/N	Absorption Band (cm^{-1})	Absorption Band Characteristics	Suspected Bond/Functional group	Inference
Humic Acid				
1	3307.71	Very strong and strongly broad	O-H (s) N-H (s)	Hydroxyl and amine groups
2	1635.16	Strong, moderately broad	C= C (s) C=O (s) N-H (b)	Hydrocarbon (alkene and aromatics), amide and carbonyl groups
3	1362.98	Very weak	C-H (b) C-N (s) N-O (s)	Substituted hydrocarbon and amine
4	529.67	Very strong and strongly broad	C-C (b) C-O (b)	Cyclic/acyclic compounds

5	1007.74	Very weak	C- O (s) C - C (s)	Alcohols, carboxylic acids. Unsaturated hydrocarbons and aromatics
Fulvic Acid				
1	3338.18	Very strong and strongly broad	O-H (s) N-H (s)	Alcohols, hydroxyl, phenols, amines
2	1634.30	Strong, moderately broad	C = C (s) C = O (s)	Alkenes, aromatics, amides, carbonyls
3	427.20	Very strong and strongly broad	C-C (b) C-O (b)	Cyclic/acyclic compounds

They are essential parameters for characterising HA and FA, influencing their properties and applications. Results are in line with key properties of HA. Total acidity influences reactivity with metals, minerals, and organic compounds. Carboxylic and phenolic groups form complexes with plant nutrient elements, enhancing bioavailability [12].

(b) Optical Characteristics: The E_4/E_6 ratio (optical characteristics) obtained for HA ranged from 4.0 to 4.3 with a mean of 4.2 ± 0.1 for HA and 5.3 to 5.9 with a mean of 5.6 ± 0.2 for FA. The E_4/E_6 ratio is a significant parameter in characterising HA and FA, which are key components of humic substances. The significance lies in the fact that it serves as (i) humification index, indicating the degree of humification, with lower values suggesting more advanced humification, a pointer to increased degree of condensation or unsaturation in the molecular fragment, (ii) molecular weight: E_4/E_6 ratio is inversely related to molecular weight; lower values indicate higher molecular weight and (iii) aromaticity: E_4/E_6 ratio reflects the aromaticity of HA and FA; lower values suggest higher aromaticity. Regarding HA, E_4/E_6 ratio between 3 and 5 indicates more humification, higher molecular weight and increased aromatic characteristics. E_4/E_6 ratio greater than 5.0, which is characteristic of FA indicates less humification, lower molecular weight and less aromatic molecular fragment. E_4/E_6 ratio influences nutrient availability and soil fertility, mobility and reactivity of HA and FA [12,13]. All these impact nutrient availability, microbial activity, and plant growth, explaining the great impact of the products in enhancing crop growth and productivity.

Table 2: Acidity groups and optical characteristics of HA and FA extracted from the EcoGrow and EcoFix

S/N	Variable	Range	*Mean	**CV (%)
Humic Acid				
1	Carboxyl acidity (meq/g)	5.3 to 6.7	6.6 ±0.9	13.6
2	Phenolic acidity (meq/g)	6.5 to 10.6	8.0 ± 1.8	22.5
3	Total acidity (meq/g)	12.2 to 18.5	14.6 ± 2.7	18.5
4	E_4/E_6 ratio	4.0 to 4.3	4.2 ± 0.1	2.4
Fulvic Acid				
1	Carboxyl acidity (meq/g)	4.7 to 5.4	4.6 ± 0.8	17.4
2	Phenolic acidity (meq/g)	6.3 to 8.2	7.4 ± 0.9	12.2
3	Total acidity (meq/g)	9.8 to 13.7	12.0 ± 2	16.7
4	E_4/E_6 ratio	5.3 to 5.9	5.6 ± 0.3	5.4

*Mean was obtained from four replicates, ** CV = Coefficient of variation,

3.2. Effect of Applied Climate - Smart, Green Chemistry Technology on Selected Soil Properties and Growth of Scotch Bonnet Pepper (*Capsicum chinense*)

Digital capture (Fig.2) revealed typical soil characteristics in the study area: strongly clayey outlook in the dry season (a) with potential to be moulded into a brick (b), characterised with high water table characteristics in the wet season (c) and the outlook of a typical regenerated and stabilised soil via the application of the innovative in-country green chemistry technology (d). The pH value for unregenerated soil ranged from 3.8 to 4.0 with a mean of 3.9 ± 0.1. Soil regeneration technology applied in this study raised the pH from 3.9 ± 0.1 to 5.9 ± 0.2 (range: 5.6 to 6.2). Based on the mean values, there was a 20-fold reduction in soil acidity by the applied green chemistry technology.

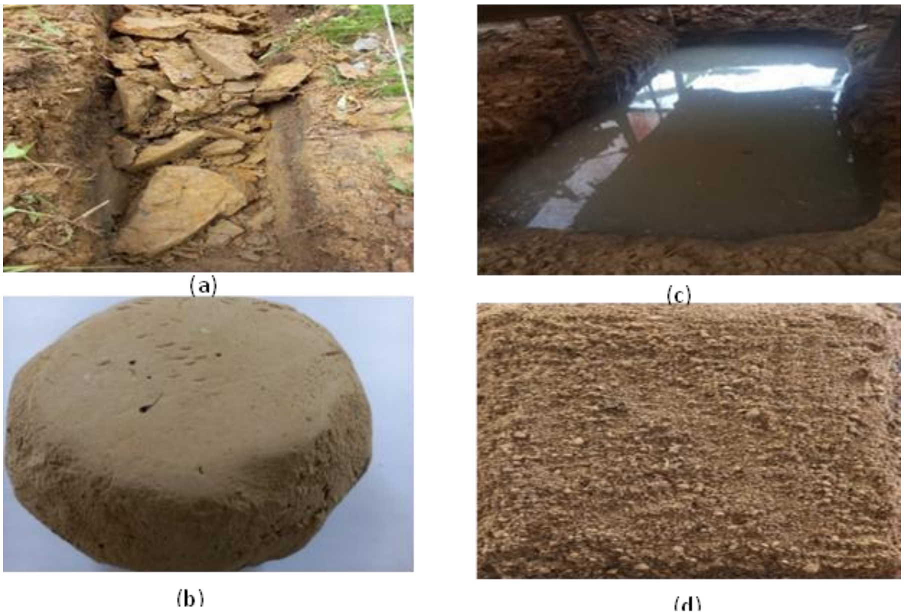


Fig.2: Typical outlooks of soil in the study are in dry (a, b) and wet season (c) versus soil regenerated by applied green chemistry technology (d)

Soil pH impacts the solubility and availability of essential plant nutrients. Maintaining pH between 6.0 and 7.0 for most crops is essential for maximizing nutrient bioavailability, fostering healthy microbial population and promoting robust plant growth. It is good to note that different crops have soil pH preferences [12]. Similarly, soil electrical conductivity (EC) ranged from 41 to 52 with a mean of 47 ± 6 for the unregenerate and 371 to 489 with a mean of 420 ± 52 for the regenerated soil, unit in μScm^{-1}, corresponding to 8.9 – fold increase based on the mean values. Soil electrical conductivity (EC) is a measure of the soils ability to conduct electrical current, which primarily depends on the concentration of soluble salts. It provides insight to soil salinity, nutrient availability and overall soil fertility. Optimal EC ranges from 200 to 1200 μScm^{-1}, ideal for most crops, promoting plant growth. Above 1200 μScm^{-1}, indicates excessive salts that can harm sensitive plants. Below 200 μScm^{-1} indicates nutrient deficiencies, a pointer to low soil fertility [7,9,2]. By implication, the soil under study was infertile (EC: 47 μScm^{-1}) but green chemistry technology applied to the soil in this study modified the EC to fall within the optimal range and restored soil fertility, thereby, promoting crop growth. Applications have been made over two years without the tendency of raising the EC above 1200 μScm^{-1} countering concerns of cumulative effect.

The trend in crop growth indices (plant height, stem girth, leaf number and canopy spread) showed vibrant and steady increase with time as presented in Fig.3. Pearson correlations between these indices and period of growth gave positive, strong and significant ($p < 0.05$) correlation coefficients, obtained as +0.990 for plant height, + 0.977 for stem girth, + 0.973 for leaf number and + 0.997 for canopy spread.

Growth rates were obtained as 1.5 cm per day for plant height, 0.04 cm per day for stem girth, 3 per day for leaf number and 0.08 cm for canopy spread.

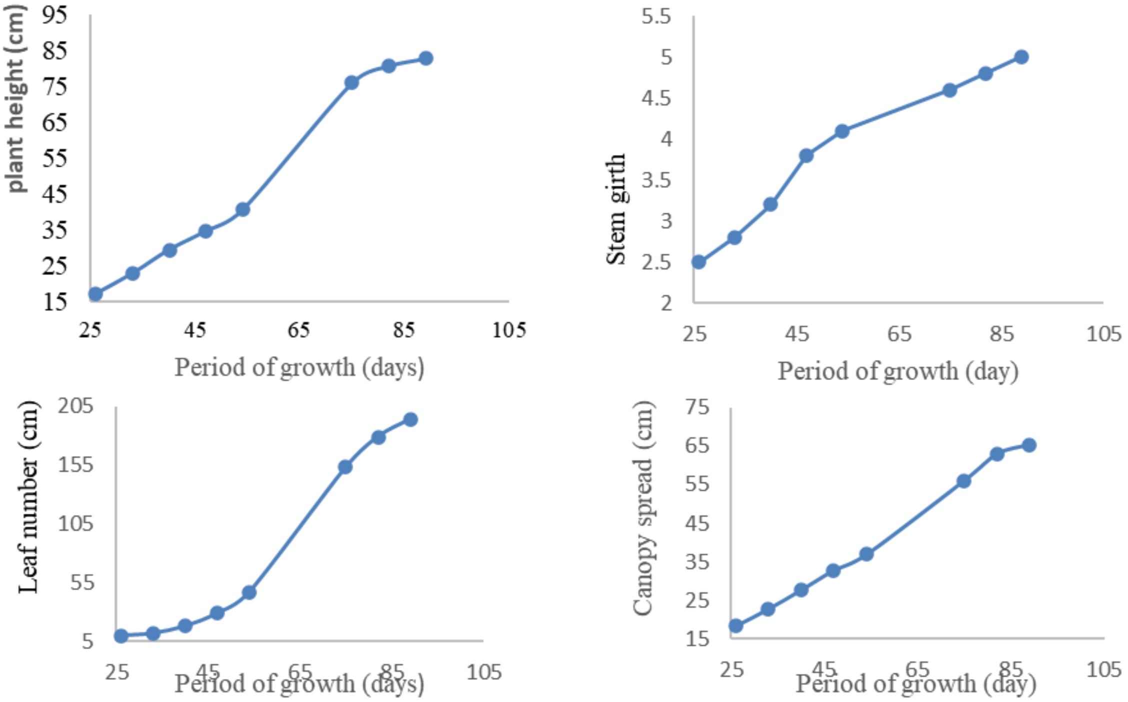
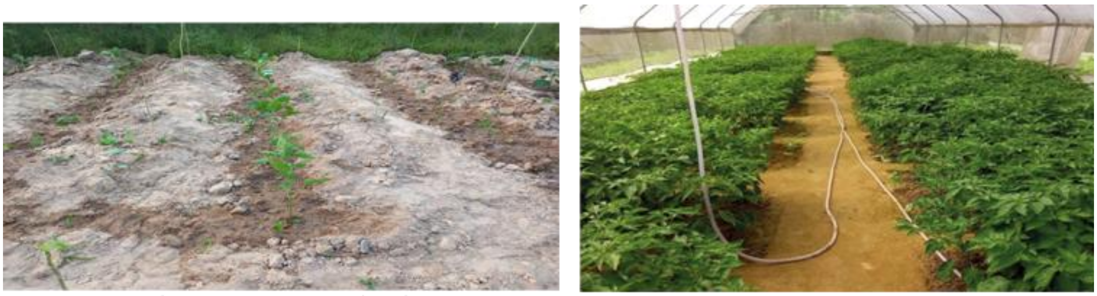


Fig.3: Growth dynamics of crops grown in green chemistry regenerated soil

A comparative evaluation between performance of Scotch Bonnet pepper grown in control (unregenerated soil) in the same project site and the crop grown in the regenerated soil (Fig.4) showed an excellent performance of the regenerated soil over the unregenerated control. Results also showed that the unripe pepper is a better source of immune boosting nutrients such as zinc (Zn): 0.58 ± 0.01 for the unripe fruit and 0.33 ± 0.01 for the ripe; Copper (Cu): 0.23 ± 0.01 for the unripe and 0.21± 0.01 for the ripe. Both elements were within acceptable limits of 0.6 for Zn and 10 for Cu (FAO,2013) with unit in mg/kg. Zinc (Zn) and copper (Cu) are essential trace minerals with key roles in the human body: zinc supports immune function, aiding in the defense against infections, is essential for wound healing and skin health, plays a role in DNA synthesis, cell division, and protein synthesis. It is important for proper growth and development, especially in children, and contributes to taste and smell. Copper is involved in energy production through the enzyme cytochrome c oxidase., supports the formation of red blood cells and maintains healthy nerves and blood vessels, contributes to iron absorption, helping prevent anemia, acts as an antioxidant, protecting cells from damage and helps in collagen production for skin, bones, and connective tissue. Crop protection management was in line with best practices [14].



Crop growth in unregenerated soil

Crop growth in green chemistry regenerated

Fig.4a: Crop growth enhancement by green chemistry versus traditional method

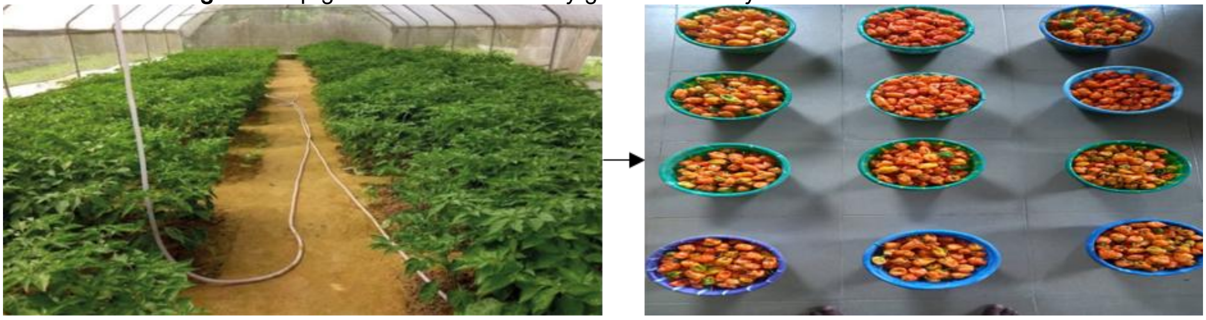


Fig.4b: Average daily harvest from crop grown in soil regenerated t by green chemistry

Fig.4c: Digital capture of scotch bonnet pepper, harvested from crops grown in soil regenerated by green chemistry technology

4. CONCLUSION AND RECOMMENDATIONS

The innovative climate smart, green chemistry-based soil regeneration technology investigated in this study offers a sustainable solution to Nigeria's food security challenges by improving soil health and fertility. By promoting resilient crop production approach, it addresses the impact of climate change and boosts crop productivity. Regeneration of coastal soil of Bayelsa by green chemistry improved soil health by positive modification of soil chemistry and improvement of soil fertility and promotion of plant growth, especially Scotch Bonnet (*Capsicum chinense*). This climate-smart technology has scalability potential and is recommended for adoption in the country to ameliorate the prevailing food security crisis. Capacity building through training of youths and small holder farmers is recommended as a drive to provide alternative economic diversification platform in Nigeria.

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BOOK OF PROCEEDINGS
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Comparison of the *in vitro* Antioxidant and Reducing Activities of Crude *Morinda lucida* Root Extracts

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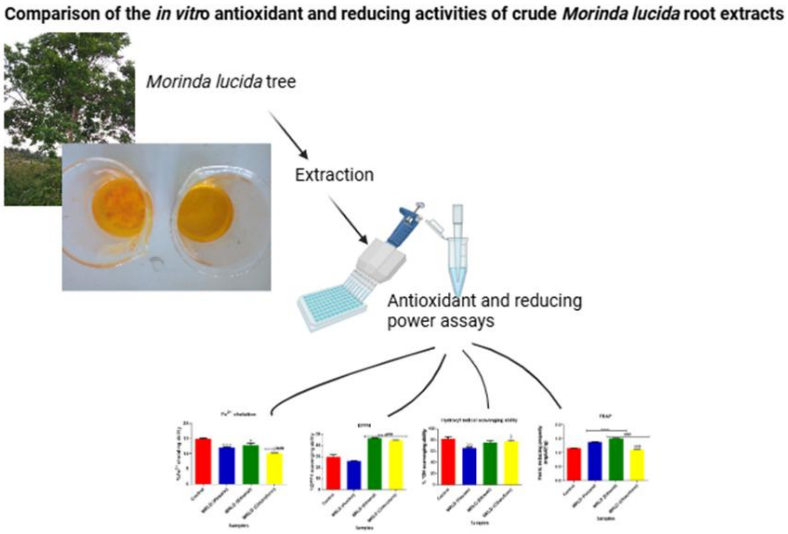
ABSTRACT

In Nigeria, one of the useful medicinal plants is *Morinda lucida.* It is widely used for medicinal purposes in Nigeria. The antioxidant and reducing properties of the crude hexane, ethanol and chloroform extracts of *Morinda lucida* were analyzed using the standard procedures for DPPH radical scavenging, ferric reducing antioxidant power (FRAP), iron chelation and hydroxyl radical scavenging were carried out using kaempferol as control. The results of this study revealed that the ethanolic extract of *Morinda lucida* significantly scavenged ($P < 0.001$) DPPH radicals when compared with the kaempferol control, the hexane and chloroform extract of *Morinda lucida*. Similarly, ethanolic extract of *Morinda lucida* significantly ($P < 0.001$) possessed the highest ferric reducing antioxidant property (FRAP) when compared with the ascorbic acid standard and other extracts of *Morinda lucida* tested. The most promising iron chelating ability was observed in the ethanolic extract of *Morinda lucida* when compared with other extracts of *Morinda lucida* tested, though the kaempferol control significantly ($P < 0.001$) had the best Fe^{2+} chelating ability. Finally, the ethanol and chloroform extracts of *Morinda lucida* significantly ($P < 0.05$) scavenged OH* radicals with no significant difference when compared with the control, while the hexane extract of *Morinda lucida* showed the lowest OH* radical scavenging ability. The assays conducted were concentration dependent with varying antioxidant potentials, hence, *Morinda lucida* could be used in the management to many forms oxidative stress related diseases, buttressing its use in folklore medicine.



KEYWORDS: Kaempferol; Chelating ability; Antioxidants; Scavenging capacity; Reactive oxygen species.

1. INTRODUCTION

The search for natural antioxidants is very important due to their potential applications in health and in food preservation. *Morinda lucida*, is a member of the Rubiaceae family, widely recognized for its extensive use in traditional medicine across various African cultures. [1-2] The roots of this plant are particularly noted for their potential therapeutic properties which are crucial in combating oxidative stress and related diseases. Recent scientific investigations have highlighted the bioactive compounds present in *Morinda lucida*, revealing a rich phytochemical profile that includes flavonoids, alkaloids, and phenolic compounds, all of which contribute to its medicinal efficacy. [3]

The significance of antioxidants in human health cannot be overstated, as they play a vital role in neutralizing free radicals and preventing cellular damage. [4] These radicals have been identified to increase the chances of various degenerative diseases and aging processes [5]. The reducing power of a compound is often correlated with its antioxidant capacity, making it an important parameter in assessing potential antioxidant agents.

The aim of this study is to compare the *in vitro* antioxidant and reducing potentials of *Morinda lucida* roots extracts, employing various assays to quantify their effectiveness. By elucidating the antioxidant potential of these extracts, this research seeks to provide insights into their pharmacological applications.

2. MATERIALS AND METHODS

2.1 Sample Collection

Morinda lucida roots was collected from Iwo Osun state, Nigeria, the roots air dried then pulverized and extracted with three solvents, hexane, chloroform and ethanol.

2.2. Chemicals and Reagents

In this investigation, analytical-grade chemicals and reagents were used. The following products were bought from Chemie GmH (Steinheim, Germany) and Sigma Aldrich, through a chemical vendor in Nigeria. Iron sulfate ($FeSO_4$), hydrogen peroxide (H_2O_2), deoxyribose, potassium ferricyanide (KFeCN), trichloroacetic acid (TCA), iron chloride ($FeCl_3$), Naphtyl ethylenediamine dihydrochloride (NEDD), 2,4-dinitrophenyl hydrazine (DNPH), and 1'1-diphenyl-2-picryl hydrazine (DPPH).

2.3 Antioxidant Parameters

1’1-Diphenyl-2-pycryl hydrazine (DPPH) test of the plant extracts was carried out as previously described Oboh[6]. The value was calculated by plotting inhibition percentages against concentrations of the plant extracts. For Reducing power, various concentrations of the extracts in 0.5 mL samples were mixed with 1 mL of a phosphate buffer (0.2 M, pH 6.6) and 1 mL of 1% potassium hexaferricyanide [$K_3Fe(CN)_6$], and the mixture was incubated at 50^{0}C for 30 min. Afterwards, 1 mL of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 3000× g for 10 min. Finally, 1 mL of the upper layer of the solution was mixed with 0.2 mL of 0.1% $FeCl_3$, the mixture was left to rest away from light and the absorbance was measured at 700 nm. The same operation was realized with BHT (0–100 μg/mL) used as a reference[7]. The antioxidant activity linked to reducing power was expressed as antioxidant power (AP) following the formula:

$$\text{AP} = \frac{Abs\ extract - Abs\ blank}{Abs\ extract}\ x\ 100.$$

The plant extracts (0.15-0.6 mM) were added to a reaction mixture containing 120 mL of 20 mM deoxyribose, 400 mL of 0.1 M phosphate buffer, 40 mL of 500 mM $FeSO_4$, and the volume were made up to 800 mL with distilled water. The reaction mixture was incubated at 37^{0}C for 30 minutes and the reaction was then stopped by the addition of 0.5 mL of 28% trichloroacetic acid[8]. This was followed by addition of 0.4 mL of 0.6% thiobarbituric acid solution. The tubes were subsequently incubated in boiling water for 20 minutes. The absorbance was measured at 532 nm in a spectrophotometer to determine the hydroxyl radical scavenging ability. [7] The Fe^{2+} chelating ability of both the plant extracts were determined using freshly prepared 500 mM $FeSO_4$ (150 mL) which was added to a reaction mixture containing 168 mL 0.1 M Tris–HCl (pH 7.4), 218 mL saline, and the plant extracts (0-0.32 mM). The reaction mixture was incubated for 5 minutes before the addition of 13 mL 0.25% 1,10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe (II) chelating ability was subsequently calculated [9] were performed to determine the antioxidant potential of various extracts of *Morinda lucida* roots using already established methods. Statistical analysis was carried out using GraphPad Prism 8.0.2 software.

3. RESULTS AND DISCUSSION

The antioxidant properties of *Morinda lucida* root extracts (MRLD) were evaluated using assays, such as DPPH scavenging activity, FRAP, Fe^{2+} chelating ability and OH* radical scavenging ability. These assays provide insight into different mechanisms of antioxidant action, allowing for a comprehensive assessment of the extracts from *Morinda lucida*. For each of the Figures (1-3), the bars represent mean ± SEM (n=3).

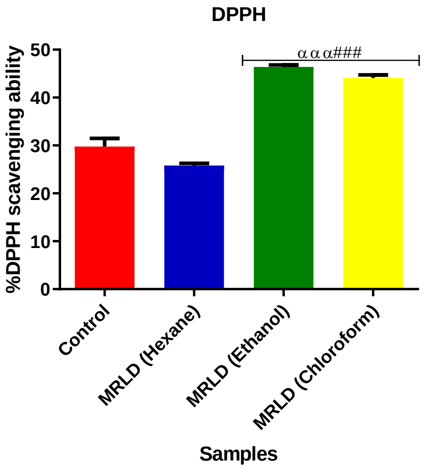


Figure 1: DPPH Scavenging ability of MRLD (Hexane, Ethanol and Chloroform) Extracts

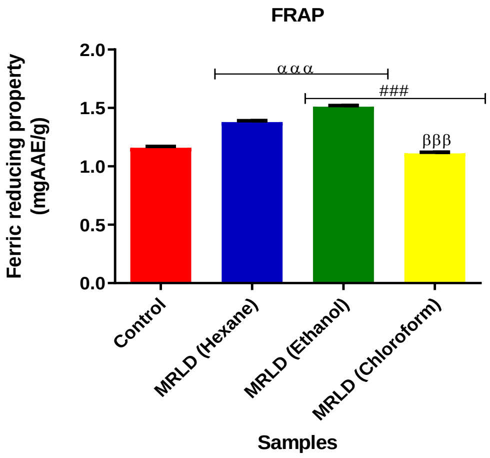


Figure 2: Ferric reducing antioxidant property of MRLD (Hexane, Ethanol and Chloroform) Extracts

3.1. DPPH Scavenging Activity

The DPPH assay results (Figure 1) indicate that the ethanol extract of *Morinda lucida* showed the best activity at 47.1 %, followed by the chloroform extract (45.6 %), while the hexane extract showed the lowest activity (26.7 %). The activities are statistically different at $^{\alpha\alpha\alpha}P < 0.001$, for kaempferol, $^{\#\#\#}P < 0.001$, for hexane as seen in Figure 1. Interestingly, both the ethanol and chloroform extracts demonstrated higher DPPH scavenging activity than the reference compound kaempferol (32.2 %). This suggests that polar and moderately polar solvents were more effective in extracting antioxidant compounds from *Morinda lucida* roots. The higher activity of the extracts compared to kaempferol may be due to the presence of a complex mixture of antioxidant compounds acting synergistically. [10]

3.2. Ferric Reducing Antioxidant Property (FRAP)

The FRAP assay as represented in milligram ascorbic acid per gram (mgAAE/g) (Figure 2) showed that the hexane, ethanol and chloroform extracts of *Morinda lucida* possessed reducing power, with the ethanol extract demonstrating the highest activity (1.53 mgAAE/g), followed by the hexane extract (1.40 mgAAE/g), and the chloroform extract (1.13 mgAAE/g). The ethanol and hexane extracts exhibited higher reducing power than the kaempferol control (1.18 mgAAE/g), indicating their potential as electron donors to neutralize free radicals. [7,9]

The result of the assay shows that they are statistically different at $^{\alpha\alpha\alpha}P < 0.001$, with Kaempferol (control),$^{\beta\beta\beta}P < 0.001$, for Ethanol, and $^{\#\#\#}P < 0.001$, for Hexane.

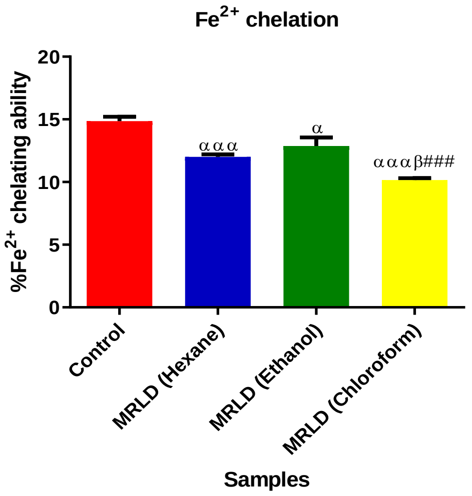


Figure 3: Fe^{2+} Chelating ability of MRLD (Hexane, Ethanol and Chloroform) Extracts

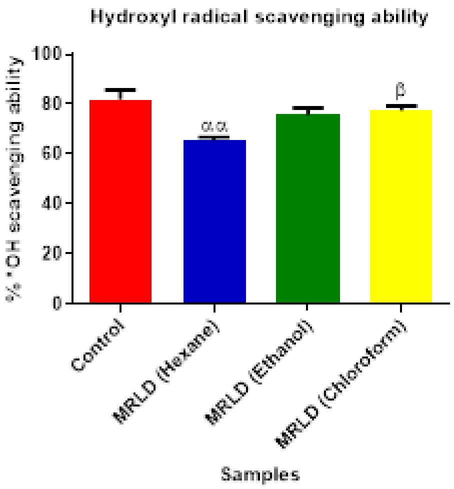


Figure 4: OH* Radical scavenging ability of MRLD (Hexane, Ethanol and Chloroform) Extracts

3.3. Fe^{2+} Chelating Ability

With respect to the chelating ability (Figure 3), the three extracts of *Morinda lucida* chelated iron but not as much as the kaempferol control, however, the ethanol extract (14.6 %) of *Morinda lucida* significantly chelated iron better than the hexane (12.6 %) and chloroform (10.5 %) extracts. The reference compound kaempferol showed slightly higher chelating ability (15.85 %). Values are statistically different at $^{\alpha\alpha\alpha}P < 0.001$, $^{\alpha}P < 0.05$ for kaempferol, $^{\beta}P < 0.05$ for Ethanol, $^{\#\#\#}P < 0.001$, for Hexane. Metal chelation is an important antioxidant mechanism, operating through metal-catalyzed reactions [11]. This result also revealed that the hexane, ethanol and chloroform extracts can scavenge hydroxyl radicals (OH*) from deoxyribose in Fenton reaction.

3.4. OH* Radical Scavenging Ability

The hydroxyl radical scavenging assay (Figure 4) demonstrated that all *Morinda lucida* extracts possessed considerable OH* radical scavenging ability. The ethanol extract had the best and most consistent activity (70.7 %), followed by the chloroform extract (73.8 %), and the hexane extract (63.7 %). Notably, kaempferol exhibited higher OH* radical scavenging ability (81.0 %). Values are statistically different at $^{\alpha\alpha}P < 0.01$, for kaempferol, $^{\beta}P < 0.05$ for Ethanol. The hydroxyl radical is one of the most reactive and damaging species in biological systems, and the ability to scavenge these radicals is crucial for preventing oxidative stress.[12]

4. CONCLUSION

The results from these antioxidant assays consistently demonstrate that the ethanol extract of *Morinda lucida* roots possesses the strongest antioxidant activity across multiple mechanisms. This suggests that polar solvents like ethanol are most effective in extracting antioxidant compounds from *Morinda lucida* roots. The varying performance of the extracts in different assays highlights the importance of using multiple methods to evaluate antioxidant potential. The strong antioxidant activity of *Morinda lucida* root extracts, particularly the ethanol extract, suggests potential applications in pharmaceuticals and nutraceuticals. However, identifying and characterizing the specific antioxidant compounds present in these extracts and their safety in biological systems is essential.

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Evaluation of the Biological Activity of *Fiscus natalensis* Leaves

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ABSTRACT

Fiscus natalensis from Moraceae family, native to Africa with edible vegetables and fruits used in traditional medicine for the treatment of illness, due its antimicrobial, anti-inflammatory, and antioxidants activities. The study aims at determining proximate analysis, phytochemical constituents, total phenolic content and antioxidant activity of the Dichloromethane and Methanol crude extracts of *F. natalensis* leaves. The phytochemical screening revealed the presence of 12 secondary metabolites in both extracts. Percentage proximate analysis showed the plant contain, Moisture content (14.44%), Dry matter content (13.12%), Ash content (9.35%), Crude Protein (5.75%), Carbohydrate (21.36%), Fat/Lipid (11.73%), and Fiber content (11.72). The total Phenolic contents were recorded as 3.82±0.15mg/g and 2.05± 0.13mg/g for DCM and Methanol crude extracts respectively. Antioxidant activity shows 74.3%, and 59.6 %, at concentration of 1.5mg/ml for DCM and Methanol extracts, against DPPH free radicals which compared well with the standard antioxidants (Butylated Hydroxyl Anisole 78.4%, Ascorbic acid 68.1%, and α-Tocopherol 63.3%). The high antioxidant activity observed in the crude extracts of this plant may be attributed to the phenolic component obtain in the results.

KEYWORDS: Anti-oxidant, *Fiscus natalensis*, Phenolic content, Phytochemical, Proximate.

1. INTRODUCTION

Fiscus nantalensis are edible fruits and vegetables used in traditional medicine to treat a variety of illnesses.[2] Alkaloids, flavonoids, tannins, saponins, and phenolic compounds are among the many bioactive secondary metabolites found in the family. Nigerian traditional healers are aware of the plant's therapeutic benefits and utilize it as herbal remedy[3]. The whole plant has been utilized for traditional medicine because of its antimicrobial, anti-inflammatory, and antioxidant properties. Carbohydrates, alkaloids, terpenoids, cardiac-glycosides, saponins, phenolic, flavonoids, anthraquinones, tannins, coumarins, resins, are secondary metabolites from plants that poses medicinal value which have biological activity against infections.[4] Natural antioxidants such as polyphenols and carotenoids are found in medicinal plant and food which shows a range of biological effects on degenerative diseases including anti-inflammatory, anti-cancer and anti-aging.[14] Studies show that plant-based antioxidants can prevent and treat degenerative diseases that are induced by oxidative stress, including diabetes, cancer, inflammation, cardiovascular disease, and dementia.[15] The research aims at investigating the proximate analysis, phytochemical composition, total phenolic content and antioxidant activities of the crude extracts of *F. natalensis* leaves.

2. MATERIALS AND METHODS

2.1. Plant collection and identification

The leaves of *Fiscus natalensis* were collected from Bomo village and Samaru Zaria. Identified and authenticated by Mr. Yahaya Umar Galla at Ahmadu Bello University, Zaria herbarium. The voucher number AB1845 and identification number NB237450 was deposited in the Herbarium ABU, Zaria. The collected sample was washed, air dry, and pulverized in to powder using a wood milling machine.

2.2. Proximate Analysis

The proximate composition (Carbohydrate, Moisture content, Crude Protein, and Ash content) of powdery sample of *F. natalensis* was determined according to standard procedures outlined by the Association of Official Analytical Chemist (AOAC, 2019).[3]

2.3. Extraction Crude Extracts

A portion (200g) of the pulverized plant was successively extracted using Soxhlet apparatus with 500ml (each) of Dichloromethane (DCM) and Methanol (MeOH) as solvents. The e fractions were collected and concentrated with the aid of a rotary evaporator at 40°C, weighed and kept at ambient temperature.

2.4. Phytochemical Screening of Crude Extract and Determination of Total Phenol Content

The fractions obtained were tested for the presence of secondary metabolites.[3] **A** critical tool for detecting the different biochemical substances produced by plants is qualitative phytochemical screening.[6]and was done using the specified standard procedures.[7]Total phenolic content of *F. natalensis* leave fractions was determined by Folin-Ciocalteu reagent [4]. The antioxidant activity of F. *natalensis* leaves fractions were determine using the DPPH free radical scavenging activity method.[8]

3. RESULTS

3.1 Proximate analysis

The results Proximate analysis of *Fiscus natalensis,* leaves *presented in table 1,* shows various nutritional and pharmacological parameter measures observed from the plant material.

Table 1: Result of Proximate Analysis of *Fiscus Proximate*.

Parameters	Percentage %
Moisture Content	17.44
Dry Matter Content	13.12
Ash Content	10.64
Crude Protein	5.75
Carbohydrate content	27.36
Fat/Lipid Content	3.97
Fiber Content	21.72

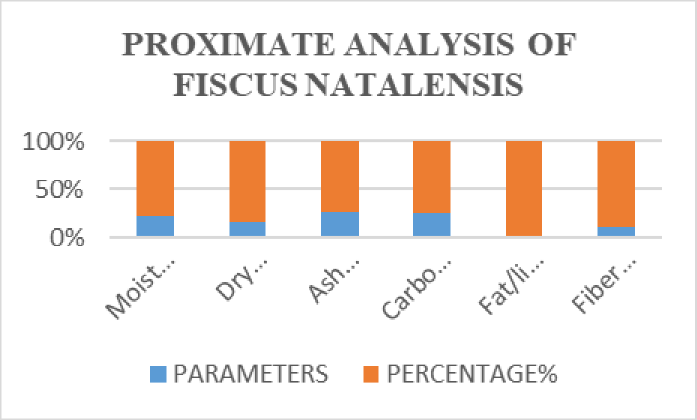

Figure 1: Percentage proximate constituent of *F. natalensis* leaves.

3.2. Phytochemical screening

Table 2 present the results of phytochemical screening of *F. natalensis, the results show* the presence nine secondary metabolites in both fractions, except for terpenoids, and cardiac glycosides which were found present in DCM fraction only.

Table 2: Result of Phytochemical screening of *Fiscus natalensis* crude extracts.

Secondary Metabolite	DCM Fraction	Methanol Fraction
Carbohydrate /reducing sugar	+	+
Alkaloids Meyer's test	-	-
Alkaloids Drangendroff's test	-	+
Terpeniod	+	-
Triterpenes	-	-
Cardiac Glycosides	+	-
Saponins	+	+
Phenolic Compounds Tannins	+	+
Flavonoids	+	+
Steroids	+	+
Quinones	-	-
Anthraquinones	+	+
Tannins	+	+
Coumarins	+	+
Amino acid	-	-
Resins	+	+

Note: "+" indicates the presence of metabolite, and "-" indicates its absence of metabolite.

3.3. Total Phenolic Content

The total phenolic content of the Methanol and DCM fractions of *F. natalensis* (Table 3) were 3.82 and 2.05 mg/g.

Table 3: Total Phenolic Content of *Fiscus Natalensis* Crude Extract

PLANT	SOLVENT	TOTAL PHENOL (mg/g)
Fiscus Natalensis	Methanol	3.82 ± 0.15
Fiscus Natalensis	DCM	2.05 ± 0.13

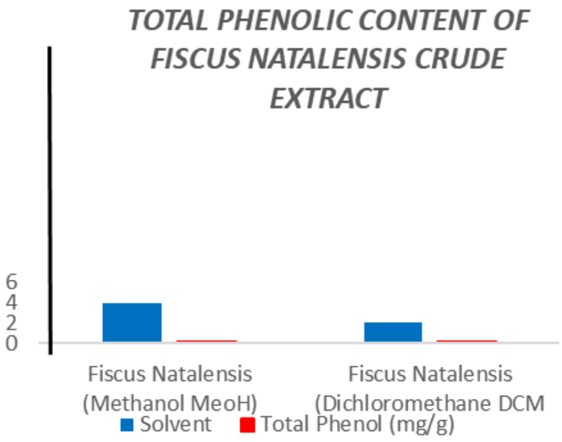

Figure 2: Total Phenolic Content of *Fiscus Natalensis* Fractions

3.4. Antioxidant Activities of *Fiscus natalensis* Fractions

Table 4 present the antioxidant activity of the DCM and Methanol fractions of *Fiscus natalensis*, the antioxidant activity compares well the standard antioxidants of Butylated Hydroxyl Anisole (BHA), ascorbic acid and α-tocopherol. The results show high activity inhibition of 74.3% against the DPPH free radicals at concentration of 1.5mg/ml.

Table 4: Absorbance and Percentage inhibition of the Fractions and Drugs Against DPPH Free Radical at 517nm Absorbance.

Plant Extracts/ Drugs	Solvent of Extraction	Concentration (mg/ml)	Absorbance	% Inhibition
Crude Extract	DCM	0.25	0.3387 ± 0.030	43.5
		0.5	0.3840 ± 0.022	55.2
		1.0	0.4344 ± 0.033	74.3
Crude Extract	Methanol	0.25	0.3387 ± 0.030	42.7
		0.5	0.3840 ± 0.022	53.4
		1.0	0.4344 ± 0.033	59.6
Ascorbic acid		0.25	0.0843 ± 0.010	50.7
		0.5	0.2883 ± 0.032	62.3
		1.0	0.3107 ± 0.142	68.1
BHA		0.25	0.0374 ± 0.023	47.2
		0.5	0.0461 ± 0.081	58.5
		1.0	0.0481 ± 0.022	78.4
α- Tocopherol		0.25	0.6801 ± 0.027	45.7
		0.5	0.7040 ± 0.021	59.4
		1.0	0.7046 ± 0.001	63.3

Key: BHA Butylated Hydroxyl Anisole

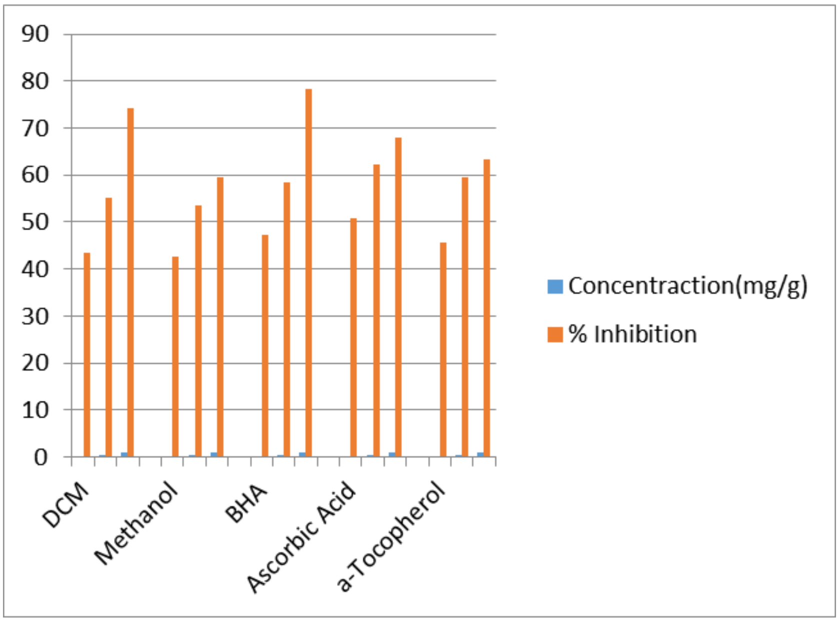

Figure 3: Absorbance and Percentage inhibition of Fractions and Drugs Against DPPH Free Radicals.

4. DISCUSSION

Proximate analysis of *Fiscus natalensis* leaves indicates that the plant contained Moisture content 17.44%, dry matter 13.12%, Ash content 10.64%, Crude Protein 5.75%, Carbohydrate 27.36%, fat/lipid content 3.97%, fibre content 21.72% respectively. A similar study presents 10.59.%, 10.83%, for ash and protein content respectively.[4,13]The fibre content of 21.72% could be of great value in both human and animal nutrition. Fibers have the potential to reduce glucose absorption as well as insulin secretion.[9] The carbohydrates content of 27.36% makes it rich in nutrients and can serve as potential nutritious food supplement.[4,13]The phytochemical screening of *F. natalensis* shows the presence of carbohydrates, alkaloids, saponins, phenolic, flavonoids, anthraquinones, tannins, coumarins, and resins as secondary metabolites in both fractions. However, terpenoids, and cardiac glycosides were found present in DCM fraction only while triterpenes, quinone and amino acid were absent in both fractions. Research has shown that tannins have the potential as an anti-inflammatory, and a means of removing harmful free radicals and reactive oxygen species.[10] Saponins are essential in diet which improve blood glucose response and antioxidant properties.[11] The phytochemicals present in *F. natalensis* leaves shows evidence of high medicinal properties therefore, it is highly recommended**.** The total phenolic content of the Methanol and DCM fractions of *F. natalensis* were 3.82 ± 0.15 mg/g and 2.05± 0.13 respectively. This indicates the quantities of phenolic compounds present in the fractions were connected with the antioxidant and anti-inflammatory properties of this medicinal plant[12].The antioxidant activity of the DCM crude extracts shows high activity inhibition of 74.3% against the DPPH free radicals at concentration of 1.5mg/ml. This result compares well with Butylated Hydroxyl Anisole (BHA) drug valuing 78.4% and water extracts of *F. natalensis leaves* valuing 74.673 ± 0.302 reported by (Peiris, *et al* 2023). The Methanol fraction have percentage inhibition of 59.6%. This shows activity inhibition but not as active as DCM fraction and is below the standard drugs (Ascorbic acid 68.1%, BHA 78.4%) but similar to that α- Tocopherol with 63.3%.

5. CONCLUSION

This research provides valuable insights in the phytochemical constituent, proximate analysis, total phenolic content and antioxidant activity of *F. natalensis* leaves. The proximate analysis provides an overview of its nutritional and pharmacological potential with high fibre and carbohydrates contents. Therefore, it can be a of a good source for foods and pharmaceuticals agents. The phytochemicals findings from this study proof its potent therapeutic qualities; therefore, the medicinal relevance is highly recommended. The research finding suggest further investigation on potential health benefits and bioactive compounds, development of functional foods and pharmaceuticals and large quantitative and qualitative study to determine correlation between antioxidant activity and total phenolic content of *F. natalensis.*

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Beningn Solvents: An Overview

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ABSTRACT

Benign solvents are environmentally friendly chemical solvents that are used as a part of green chemistry and can as well be known as green solvents. They came to prominence in 2015, when the UN defined a new sustainability-focused development plan based on 17 sustainable development goals, recognizing the need for green chemistry and benign solvents for a more sustainable future. They are developed as more environmentally friendly solvents, derived from the processing of agricultural crops or otherwise sustainable methods as alternatives to petrochemical solvents. The conventional solvents used in chemical, pharmaceutical, biomedical and separation processes represent a great challenge to green chemistry because of their toxicity and flammability. Water has been the most popular choice so far, followed by ionic liquids, surfactant, supercritical fluids, fluorous solvents, liquid polymers, bio-solvents and switchable solvent systems. Herein, we review the literature published in recent years on the classifications, characteristics, uses, pros and cons of benign solvents in various academic and industrial fields. There is a need to use eco-friendly benign solvents in various industrial applications to help minimize environmental pollution and economic cost.

KEYWORDS: Benign Solvent, Ionic Liquids, Supercritical Fluids, Sustainable, Environmental Pollution.

1. INTRODUCTION

Benign solvents are less harmful and less toxic green solvents used in place of petrochemical based solvents in order to reduce their harmful and toxicity effect to the environment and human health. Benign solvent is a reaction medium for different synthesis processes.[1] Most recently, petrochemical based solvents were the most common and perhaps the only choices of solvents among chemists. Some of them pose adverse effect on the human health and increase environmental cost.[2] They are extensively used in organic synthesis and a matter of much concern due to their characteristics such as high flammability, volatility and toxicity.[3] This scenario has been changed due to the intensive research towards environmentally benign substitutes for volatile and toxic organic solvents. Benign solvents include supercritical fluids, ionic liquids, polyethylene glycol, low melting polymers, perfluorinated solvents, deep eutectic solvents and water etc. Now chemists have to deal with the challenges of reducing the environmental impact of the processes without losing their efficiency by using these benign solvents under the concepts of green chemistry, this has emerged as an important area of chemistry and has achieved outstanding progresses towards the development of benign reaction processes.[4]

2. CLASSIFICATIONS, EXAMPLES AND USES OF BENIGN SOLVENTS

Benign solvents can be classified based on their chemical nature and sources, they include

2.1. Bio-Based Benign Solvents

These are environmentally friendly solvents derived from renewable biological resources such as plants, animals, or microbes. Unlike traditional solvents, which are typically derived from petrochemical sources, bio-based solvents are made from naturally occurring substances, which makes them more sustainable and less harmful to the environment. They are increasingly being used in industrial and pharmaceutical applications as alternatives to volatile organic compounds (VOCs) and other hazardous solvents.[5]

(a). Characteristics of Bio-Based Benign Solvents:

These solvents come from renewable feed stocks like corn**,** soybeans**,** sugarcane and cellulosic biomass**,** it reducies the dependence on fossil fuels. Bio-based benign solvents are generally less toxic than conventional solvents, making them safer for both human health and the environment. They break down more easily in the environment, reducing the risk of long-term pollution. Bio-based benign solvents are applied in various industries in producing cleaning products, coatings materials, pharmaceuticals, agrochemicals, and as alternatives to conventional solvents in chemical processes.[6]

(b). Examples of Bio-based Solvents:

- **Ethanol**: This is produced from the fermentation of sugars in crops like corn or sugarcane.
- **Lactic Acid**: This is derived from the fermentation of biomass and used as a solvent in cosmetics, pharmaceuticals, and food industries.
- **Glycerol**: A bye-product of biodiesel production, used in many personal care and pharmaceutical products.
- **D-Limonene**: This is extracted from citrus fruits like orange and lime. It is often used as a natural solvent in cleaning products and degreasers.[7]

(c). Applications of Bio- Based Benign Solvents

Bio-based benign solvents are used in industries that prioritize sustainability, such as

- **Green Chemistry**: In the production of environmentally friendly chemical processes.
- **Cleaning Products**: As solvents in biodegradable cleaning agents.
- **Paints and Coatings**: To reduce the environmental impact of volatile organic compounds (VOCs) emissions from traditional paints.[7]

(d). Associated Risk of Bio- Based Benign Solvents

- **Cost and Performance**: Bio-based benign solvents offer environmental benefits however they are very expensive to produce and sometimes having varied performance characteristics compared to petrochemical-based solvents.[8]

2.2. Supercritical Fluids

Supercritical Fluids such as *supercritical* CO_2, are substances that exist at temperatures and pressures above their critical point, combining properties of gases and liquids. It is widely recognized for its non-toxic, non-flammable characteristics and its ability to replace harmful organic solvents in extraction processes.[7] Supercritical CO_2 is being increasingly used in green extraction processes for natural compounds like essential oils and bioactive molecules, offering an eco-friendly alternative to traditional solvent extraction.

(a). Key Characteristics of Supercritical Fluids (SCFs):

1. **Density Similar to Liquids**: SCFs can dissolve solutes effectively, acting as solvents for a wide range of substances.

2. **Viscosity and Diffusion Rates Similar to Gases**: They can penetrate porous materials more efficiently than liquids, enhancing extraction processes.
3. **Tunable Solvent Properties**: By adjusting temperature and pressure, the solubility of substances in supercritical fluids can be finely controlled.
4. **Environmentally Friendly**: SCFs, especially supercritical CO_2 ($scCO_2$), are non-toxic, non-flammable, and can replace harmful organic solvents in industrial applications.[9]

(b). Applications of Supercritical Fluids:

- **Extraction**: SCFs are commonly used for extracting flavors, fragrances, and pharmaceuticals, particularly in the food and pharmaceutical industries.
- **Chemical Reactions**: Supercritical fluids are used in chemical synthesis, catalysis, and polymerization, where their tunable properties improve efficiency and selectivity.
- **Green Chemistry**: Due to their minimal environmental impact, SCFs are integral to green chemistry initiatives that seek to reduce the use of hazardous solvents.[10]
- **Nanoparticle Synthesis**: SCFs are employed in producing nanomaterials due to their ability to dissolve and precipitate materials at controlled rates, resulting in uniform particle sizes.
- **Pharmaceutical and Food Processing**: Advances in supercritical fluid technology have enhanced its use in pharmaceutical crystallization and food processing, where it helps maintain product purity and reduce toxic residues.[11]

2.3. Ionic Liquids (ILs)

Ionic liquids are salts in a liquid state at room temperature. Due to their negligible vapor pressure, thermal stability, and tunable properties, they are considered green solvents. Common examples include 1-Butyl-3-methylimidazolium chloride.[6,12,14]

2.4. Deep Eutectic Solvents (DESs)

Deep eutectic solvents (DESs) are formed by mixing hydrogen bond donors and acceptors, leading to lower melting points. They are biodegradable, non-volatile, and less expensive to produce. They include choline chloride and urea mixtures which are used in various applications like metal extraction and biocatalysis.[15] It was first introduced in 2001 and obtained its name in 2003 having considered to be one of the most innovative products ever made in history, leading to massive advancements in all industries.[16] Due to their many similar properties to ILs, such as high thermal stability, low vapor pressure, and volatility, DESs are sometimes known to be a classification of ionic liguids (ILs). When comparing ILs to DESs, DESs have the benefit of being easily synthesize since they only require mixing, and no further purifications are needed, additionally they have a low production cost. On the other hand, the high viscosity and solid state of DESs at room temperature may be a little bit of an issue. However, the physicochemical features of DESs can be tailored by carefully selecting the suitable hydrogen bond acceptor and donor.[16]

2.5. Water as a Benign Solvent

Water is one of the most benign and sustainable solvents that is non-toxic, non-flammable, and readily available. Many chemical reactions, especially enzymatic processes, are now being optimized to take place in aqueous environments to reduce reliance on hazardous organic solvents.[7] The term "universal solvent" was coined for water because of its ability to form a solution with almost any substance.[17] Its molecular structure has both positive and negative sides, so it effectively binds with other molecules of the opposite charge. However, despite being the so-called solvent for all, it is highly regarded as the greenest solvent as it possesses the following qualities: abundant, natural, low-cost, ubiquitous, and ready for use.[17] The unique characteristics of water make it distinct from any other solvent. Their high heat capacity allows it to absorb heat from its surroundings and then dissipate it as a cooling effect under extreme exothermic conditions. As a desirable heat buffer, it maintains a persistent state by resisting change. In terms of acting as a solvent, it improved the chemoselectivity and regioselectivity of solutions while increasing the rate of the chemical reaction.[18]

3. GENERAL CHARACTERISTICS OF BENIGN SOLVENTS

Benign solvents which can as well be referred to as green or environmentally friendly solvents possess several key characteristics that distinguish them from traditional hazardous solvent.

These properties are the brain behind benign solvents playing a very important role in sustainable chemistry, contributing to greener industrial processes and the transition to a circular economy which aligns with the UN sustainable development goals.[19]

They possess these outlined characteristics.

1. **Low Toxicity**: These solvents are friendly to human health and the environment. They are designed to minimize exposure to harmful chemicals, unlike petrochemical based solvents such as toluene and chloroform.[8]
2. **Biodegradability**: Benign solvents are biodegradable, it poses no trait to the environment thereby minimizing soil and water contamination.
3. **Low Volatility**: These solvents often have lower vapor pressures, this characteristics minimizes the emission of volatile organic compounds (VOCs) that contribute to air pollution.[3]
4. **Renewable Sources**: Most of the benign solvents such as bio-based solvents and deep eutectic solvents (DESs) are derived from renewable resources like glycerol or other organic matter, reducing reliance on petrochemicals.
5. **Reusability and Recyclability**: Benign solvents are often designed to be recyclable within processes, which reduces waste and energy consumption during manufacturing.
6. **Reduced Environmental Impact**: These solvents aim to lower the carbon footprint and decrease the overall environmental damage during production, use, and disposal.[9,18]

4. CONCLUSION

The shift towards benign solvents represents an important advancement in the pursuit of sustainable chemistry. The chemical industry can significantly reduce its ecological footprint by replacing traditional petrochemical solvents with environmentally friendly alternatives such as bio-based solvents, supercritical fluids, ionic liquids, deep eutectic solvents, and water,. These solvents not only offer lower toxicity and enhanced biodegradability but also minimize the emission of volatile organic compounds (VOCs), thereby contributing to cleaner air and safer working environments. The classification of benign solvents highlights the diverse options available for various applications from extraction processes to chemical synthesis. The unique properties of each category, such as the tunable characteristics of supercritical fluids and the non-volatility of ionic liquids, enhance their utility across numerous industries, including pharmaceuticals, agrochemicals, and materials science. However, challenges remain in terms of cost and performance, particularly for bio-based solvents, which may not always match the efficiency of their petrochemical counterparts. Ongoing research and development are essential to optimize these alternatives, ensuring they can compete in a market where traditional solvents have been entrenched for decades. In conclusion, the development and implementation of benign solvents align with the principles of green chemistry and sustainability, offering promising solutions for reducing environmental impact and carbon footprint while maintaining the efficacy of chemical processes. The commitment to utilizing these innovative solvents is crucial for the transition to a more sustainable chemical industry and achieving broader environmental goals.

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Histidine-Derived Sulphonamides Bearing Carboxamide Functionality: Synthesis, *In Silico* Molecular Docking, and *In Vitro* Pharmacological Activities

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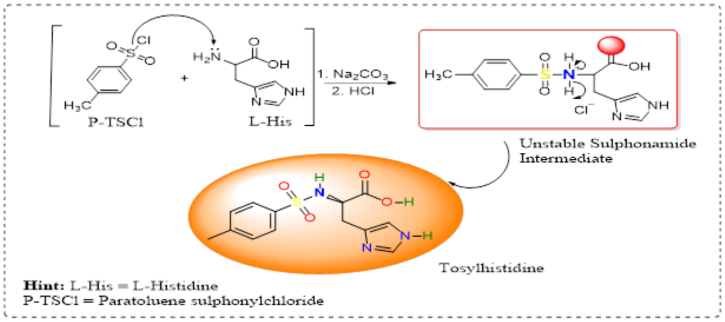
ABSTRACT

The synthesis of histidine-derived sulphonamides bearing carboxamides' functionality as potential antimalarial and antioxidant agents has been reported. The reaction between para–toluene sulphonyl chloride (a) and L-histidine (b) in a basic solution afforded tosyl histidine intermediate (c), which was then coupled with various secondary amines to obtain the corresponding carboxamides (d – h). The structure of the produced compounds was elucidated using Fourier Transform Infrared (FTIR), Proton, and Carbon -13 (^{1}H-NMR and ^{13}C-NMR) Nuclear Magnetic Resonance Spectroscopic techniques, and subsequently underwent *in vitro* and *in silico* molecular docking evaluations for antimalarial and antioxidant activities, demonstrating favorable results. The result from the docking studies indicates that compound d (-7.8 kcal/mol) would be a better antimalarial agent than artesunate (-7.4 kcal/mol), while compound g (-8.0 kcal/mol) outperformed ascorbic acid (-6.5 kcal/mol) as an antioxidant agent. Similarly, the *in vitro* antimalarial and antioxidant activity evaluation confirmed compounds (d and g) to be the most potent antimalarial agent, while compound g displayed the most promising antioxidant activity. The synthesized compounds have been confirmed to possess the potential to function as antimalarial and antioxidant agents.



KEYWORDS: Histidine, Sulphonamides, Carboxamides, Antimalarial, Antioxidant, Molecular Docking,

1. INTRODUCTION

Metabolic processes and environmental pollutants are the primary sources of free radicals in the human body[1]. This can lead to various diseases, including malaria, cancer, heart disease, premature aging, inflammation, and reduced immunological function, rendering the body more vulnerable to infections[2-4]. Various plasmodium parasites, including *falciparum, vivax, malariae, ovale, and knowlesi* are the major causative agent of Malaria[5]. Sulphonamides and their carboxamides analog are valuable medicinal molecules due to their low toxicity and outstanding inhibition potential[6]. They have been established to exhibit a range of pharmacological activities, such as antimalarial[7], anticonvulsant[8], antihypertensive[9], anthelmintic[10], and HIV Protease inhibitors[11], among others. The World Health Organization's 2018[12], malaria report indicated approximately two hundred and nineteen (219) million cases of the disease in eighty-seven (87) countries in 2017, resulting in four hundred and thirty-five thousand (435,000) fatalities, predominantly among women and children in Africa. Despite a period of remarkable success in the war against malaria, progress appears to have slowed down. This is attributed to the parasite's rising resistance to already available antimalarial drugs. Incorporating bioactive amino acids like serine and methionine into the sulphonamide structure is a viable strategy for addressing malaria-resistant parasites and improving biological efficacy[13]. This study combined biologically active amino acids like L-histidine with bioactive sulphonamide compounds containing carboxamide functionality into a single pharmacophore. It assessed their effectiveness against malaria-resistant parasites and their ability to reduce oxidative stress. The synthesized compounds demonstrated effective inhibition against the parasite development due to their synergistic activity.

2. MATERIALS AND METHODS

2.1. Instrumentation

All analytical grade reagents were purchased from Bristol Scientific Company Limited Liverpool Road, Apapa, Lagos, Nigeria, and used without further purification. Infrared spectra were obtained using a PerkinElmer FTIR spectrophotometer with potassium bromide. Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) Spectroscopy was determined using Jeol 400MHz at Rhodes University, Faculty of Pharmacy, Artillery Road, Makhanda 6139 Grahamstown, South Africa. Chemical shifts were given in parts per million (ppm), reported in delta (δ) scale, and referenced to tetramethylsilane. The Biochemistry Department of the University of Nigeria, conducted the *in vitro* activity evaluation while the Department of Chemistry conducted the molecular docking investigation.

2.2. Method of synthesis

2.2.1. Synthesis of tosyl histidine (c)

Sodium trioxocarbonate (IV) (Na_2CO_3) 2.5 g (12 mmol) was added to a suspension of L-histidine 2.0 g (b) (10 mmol) in water (H_2O) (12 ml). The clear solution was cooled to -5°C using an ice pack and para-toluene sulphonyl chloride (a) 2.2 g (12 mmol) was added in four batches over an hour. The mixture was then adjusted to room temperature and left to stand for about four (4) hours while continuously stirred with a magnetic stirrer. Upon completion of the reaction, it was acidified with 20% concentrated hydrochloric acid solution to a potential of Hydrogen (pH) of 2.0 and left undisturbed for about 12 hours for the crystallization process to proceed to completion. The crystallized crude product was then filtered via suction, washed with a buffer solution with a potential of Hydrogen (pH) of about 2.2, and dried in a desiccator to afford tosyl histidine (c).

2.2.2. Synthesis of L-histidine-based carboxamides derivatives (d – h)

To a solution of tosyl histidine intermediate (0.74 mmol) in dichlormethane (10 ml) was added triethylamine (1.11 mmol), *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide.hydrochloride (0.93 mmol) and 1-hydroxybenzotriazole (0.74 mmol) at 0°C. After stirring for 15 minutes, the corresponding amine (0.80 mmol) was added and stirred at the same temperature for about one hour. The resulting mixture was adjusted to room temperature and stirred for 19-20 hours as monitored with a Thin Layer Chromatographic plate (TLC). Upon completion of the reaction, it was diluted with Dichloromethane (DCM), and washed with 50 milliliters of water twice to obtain the aqueous and organic layer. The organic layer was then purified with 1Molar HCl (50 ml), 5% $NaHCO_3$ (50 ml), and brine solution (50 ml), and dried over Na_2SO_4 to obtain the crystallized compounds (d – h) which were further analyzed.

2.3. In silico Studies

2.3.1. Molecular docking

Molecular docking studied two human diseases, antimalarial and antioxidant. The drug target selected for antimalarial was *Lactate dehydrogenase* (PDB entry: 1LDG) while the drug target for antioxidants was *Catalase* (PDB entry: 1TGC). The 3-dimensional structures of the drug target were downloaded from the protein data bank (PDB), (http://www.pdb.org) database, and loaded into the molecular operating environment. Similarly, the standard drugs ascorbic acid (antioxidant) and artesunate (antimalarial) were obtained from PubChem. The co-crystallized ligand was removed from the corresponding protein structures. Hydrogen atoms were introduced to the proteins after the water molecules were extracted. The ligands were created, optimized, and then docked after choosing the X, Y, and Z center coordinates and grid dimensions. Image preparation and docking were conducted using AutoDock Vina (Scripps Research Institute, San Diego, California, USA), BIOVIA Discovery Studio, and molecular visualization software such as PyMOL to generate 3D structures.

2.3.2. Antimalarial inhibitory activity by Lactate Dehydrogenase (LDH) method

The synthetic compounds' *in vitro* antimalarial efficacy was evaluated by inhibiting the produced parasite protein lactate dehydrogenase (pLDH) according to the method described by Borgati[14]. The assay mixture contained 1.7 ml of tris (phosphate) buffer, 1.0 ml of 1.8 nm sodium pyruvate, 0.1 ml of 5 mmol Nicotinamide adenine dinucleotide (NAD) + hydrogen (H), and 0.1 ml of 0.5 mM of five (5) synthesized compounds (d - h). The chemical reaction was initiated by adding 0.1 ml of LDH extract and a spectrophotometric microplate reader was used to read the absorbance of 1.316 at 340 nm. The standard drug, artesunate was also prepared with the same method. The last stage of glycolysis involves the interconversion of pyruvate to lactate, which is catalyzed by the enzyme, Lactate Dehydrogenase.

Percentage inhibition was calculated using the formula thus:

$$\%\ inhibition = [\frac{\mathrm{Af} - \mathrm{Ai}}{\mathrm{Af}}\ \mathrm{X100}]$$

Where A_f = initial absorbance of 1.316 nm at 340 nm A_i = final absorbance of the synthesized compounds

2.3.3. Antioxidant activity by 2,2-diphenyl-1-picryhydrazyl (DPPH) method

The *in vitro* DPPH antioxidant activity of the synthesized compounds (d – h) was investigated by their ability to inhibit the generated stable free radicals according to the standard protocol reported by Sribalan[15]. 2 mg of DPPH was dissolved in 10 ml of methanol to prepare the stock solution. The solution was filtered, yielding a useable combination with an absorbance of 0.973 at 517 nm. After adding 1 mL (0.5 mM) of the synthesized compounds to 3 mL of DPPH useable mixture in a test tube, it was left to settle at room temperature for half an hour in the absence of light. Ascorbic acid was similarly prepared by mixing 1 mL DPPH solution with double distilled water. The absorbance was determined using a spectrophotometer as the purple color of DPPH turned yellow by accepting hydrogen from the antioxidants at 517 nm. The percentage of DPPH radical scavenging activity was calculated with the formula thus;

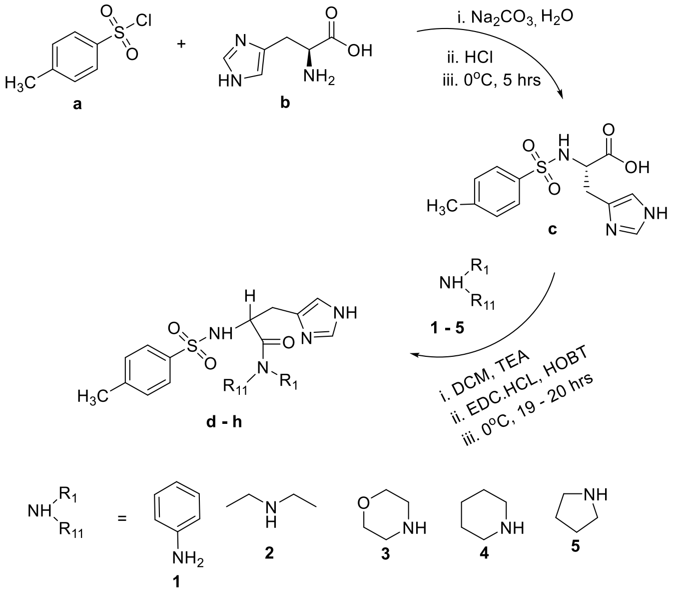
$$\%\ \text{of DPPH activity} = [\frac{Ac-As}{Ac}]\ \mathrm{x}\ 100$$

Where Ac = control reaction absorbance and As = sample absorbance

3. RESULTS AND DISCUSSION

3.1. Results

The reaction of para-toluene sulphonyl chloride (a) with L-histidine (b) yielded tosyl histidine (c) which had the acid functionality subsequently amidated with five (5) secondary amines by nucleophilic substitution reaction to obtain the corresponding carboxamides (d – h).



Scheme 1: The synthetic route

3.1.1. Tosylhistidine (Intermediate) (c)

Appearance: white solid, M.P: 195 – 196°C, FTIR (KBR) cm^{-1} 3476, 3444 (N-H band), 3137 (OH of COOH), 3096, 3029 (C-H aromatic), 2981, 2954 (C-H aliphatic), 1729 (assigned to C=O of COOH), 1535, 1496 (C=C aromatic), 1187, 1122 (2S=O).

3.1.2. 3-(1H-imidazol-4-yl)-2-((4-methylphenyl) sulfonamido)-N-phenylpropanamide (d):

Appearance: thick oily black liquid, yield 1.62 g (78.6%).
FTIR (KBR) cm^{-1} 3674, 3463, 3299 (N-H), 3187 (C-H aromatics), 2981, 2789 (C-H aliphatic), 1284 (S=O), 1620 (C=O), 1071 (SO_2NH).
^{1}H NMR (DMSO-d_6) δ: 9.60 (s, 1H, NH-C=O), 8.7 (s, 1H, CH=N), 8.3 (s, 1H, NH-SO_2), 7.7 (s, 1H, CH-NH), 7.7-7.1 (d, 9H, Ar-H), 4.8 (t, 1H, CH-CH_2), 3.0 (d, 2H, CH_2-CH), 2.4 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 173.0 (C=O), 142.33, 140.89, 136.40, 129.63, 128.99, 127.26, 125.29, 121.70, 120.35, 109.33 (aromatic carbons), 77.35, 28.50, 21.53 (aliphatic carbons).

*3.1.3. N,N-*diethyl-3-(1*H*-imidazol-4-yl)-2-((4-methylphenyl)sulfonamido)propanamide (e)

Appearance: dark brown solid, yield 0.83 g, (75.4%), M.P: 149 – 150°C.
FTIR (KBR) cm^{-1} 3567, 3395 (N-H), 3100 (C-H aromatics), 2853, 2705 (C-H aliphatic), 1618 (C=O), 1247 (S=O).
^{1}H NMR (DMSO-d_6) δ: 13.2 (HN-CH=N), 8.2 (s, 1H, CH=N), 7.7 (s, 1H, HN-SO_2), 7.7-7.4 (d, 4H, Ar-H), 7.60 (s, 1H, CH-NH), 3.9 (t, 1H, CH-CH_2), 3.3 (q, 2H, CH_2-CH_3), 2.4 (d, 2H, CH_2-CH), 2.3 (s, 3H, CH_3), 1.2 (t, 3H, CH_3-CH_2).
^{13}C-NMR (DMSO-d_6) δ: 165.0 (C=O), 142.91, 137.44, 129.60, 127.05, 125.07, 120.21, 109.42 (aromatic carbons), 76.71, 42.00, 32.00, 21.48, 14.14 (aliphatic carbons).

3.1.4. N-(3-(1*H*-imidazol-4-yl)-1-morpholino-1-oxopropan-2-yl)-4 methylbenzenesulphonamide (f)

Appearance: light yellow oily liquid, yield 1.16 g (63.7%).
FTIR (KBR) cm^{-1} 3596, 3546, 3347 (N-H), 3243 (C-H aromatics), 2864, 2697 (C-H aliphatic), 1267 (S=O), 1631 (C=O), 1126 (SO_2NH).

^{1}H NMR (DMSO-d_6) δ: 8.2 (s, 1H, CH=N), 7.8 (s, 1H, HN-SO$_2$), 7.7 (s, 1H, CH-NH), 7.7-7.3 (d, 4H, Ar-H), 3.9 (t, 1H, CH-CH$_2$), 3.6 (t, 4H, CH$_2$-O-CH$_2$), 3.1 (t, 4H, CH$_2$-N-CH$_2$), 2.5 (d, 2H, CH$_2$-CH), 2.1 (s, 3H, CH$_3$).
^{13}C-NMR (DMSO-d_6) δ: 165.0 (C=O), 143.97, 132.06, 129.76, 128.70, 125.07, 120.21, 109.42 (aromatic carbons), 77.35, 76.71, 66.11, 46.00, 21.56 (aliphatic carbons).

3.1.5. ***N*-(3-(1*H*-imidazol-4-yl)-1-oxo-1-(piperidin-1-yl)propan-2-yl)-4 methylbenzenesulphonamide (g)**

Appearance: light brown oily liquid, yield 1.53 g (84.1%).
FTIR (KBR) cm^{-1} 3597, 3426 (N-H), 3072 (C-H aromatic) 2933, 2762 (C-H aliphatic), 1612 (C=O), 1458 (C=C aromatics), 1310 (S=O), 1117 (SO$_2$NH).
^{1}H NMR (DMSO-d_6) δ: 13.5 (s, 1H, HN-CH=N), 8.6 (s, 1H, HC=N), 8.2 (s, 1H, HN-SO$_2$), 7.8 (s, 1H, CH-NH), 7.6-7.3 (d, 4H, Ar-H), 4.3 (t, 1H, CH-CH$_2$), 3.5 (t, 4H, CH$_2$-N-CH$_2$), 2.9 (d, 2H, CH$_2$-CH), 2.4 (s, 3H, CH$_3$), 1.5 (t, 6H, CH$_2$-CH$_2$-CH$_2$).
^{13}C-NMR (DMSO-d_6) δ: 169.0 (C=O), 143.30, 140.56, 133.27, 129.03, 128.50, 127.77, 117.96 (aromatic carbons), 76.72, 46.94, 30.94, 25.17, 23.53, 21.52 (aliphatic carbons).

3.1.6. ***N*-(3-(1*H*-imidazol-4-yl)-1-oxo-1-(pyrrolidin-1-yl)propan-2-yl)-4-methylbenzenesulphonamide (h)**

Appearance: sticky ox-blood solid product, yield 1.40 g (81.0%), M.P: 119 – 120^{O}C.
FTIR (KBR) cm^{-1} 3486, 3395 (N-H), 3096 (C-H aromatic) 2873, 2761 (C-H aliphatic), 1628 (C=O), 1489 (C=C aromatics), 1287 (S=O), 1123 (SO$_2$NH).
^{1}H NMR (DMSO-d_6) δ: 8.4 (s, 1H, HC=N), 8.2 (s, 1H, NH-SO$_2$), 7.8 (s, 1H, CH-HN), 7.7-7.3 (d, 4H, ArH), 4.1 (t, 1H, CH-CH$_2$), 3.5 (t, 4H, CH$_2$-N-CH$_2$), 2.9 (d, 2H, CH$_2$-CH), 2.4 (s, 3H, CH$_3$), 1.7 (t, 4H, CH$_2$-CH$_2$).
^{13}C-NMR (DMSO-d_6) δ: 172.0 (C=O), 143.31, 136.64, 133.99, 129.61, 128.96, 127.60, 125.80 (aromatic carbons), 54.19, 47.91, 37.96, 25.20, 21.52 (aliphatic carbons).

3.2. Molecular Docking Evaluation

Table 1: Results of *in silico* antimalarial and antioxidant activities evaluation*

Table 1, below shows the binding affinity of the five (5) synthesized compounds and standard drugs against the enzyme, *Lactate dehydrogenase for antimalarial activity evaluation and the enzyme, catalases for antioxidant activity evaluation respectively*. The surface interaction of the synthesized compounds against the enzymes and the amino acid residues were further illustrated in **Figure 1**.

Compounds	**Antimalarial**	**Antioxidant**
	3LDH	2CAS
d	-7.8	-7.3
e	-6.3	-6.8
f	-7.0	-7.0
g	-7.5	-8.0
h	-6.8	-7.2
Standard	-7.4	-6.5

*3LDH and 2CAS are drug targets for antimalarial and antioxidant
Standard drugs for 3LDH = artesunate; 2CAS = ascorbic acid*

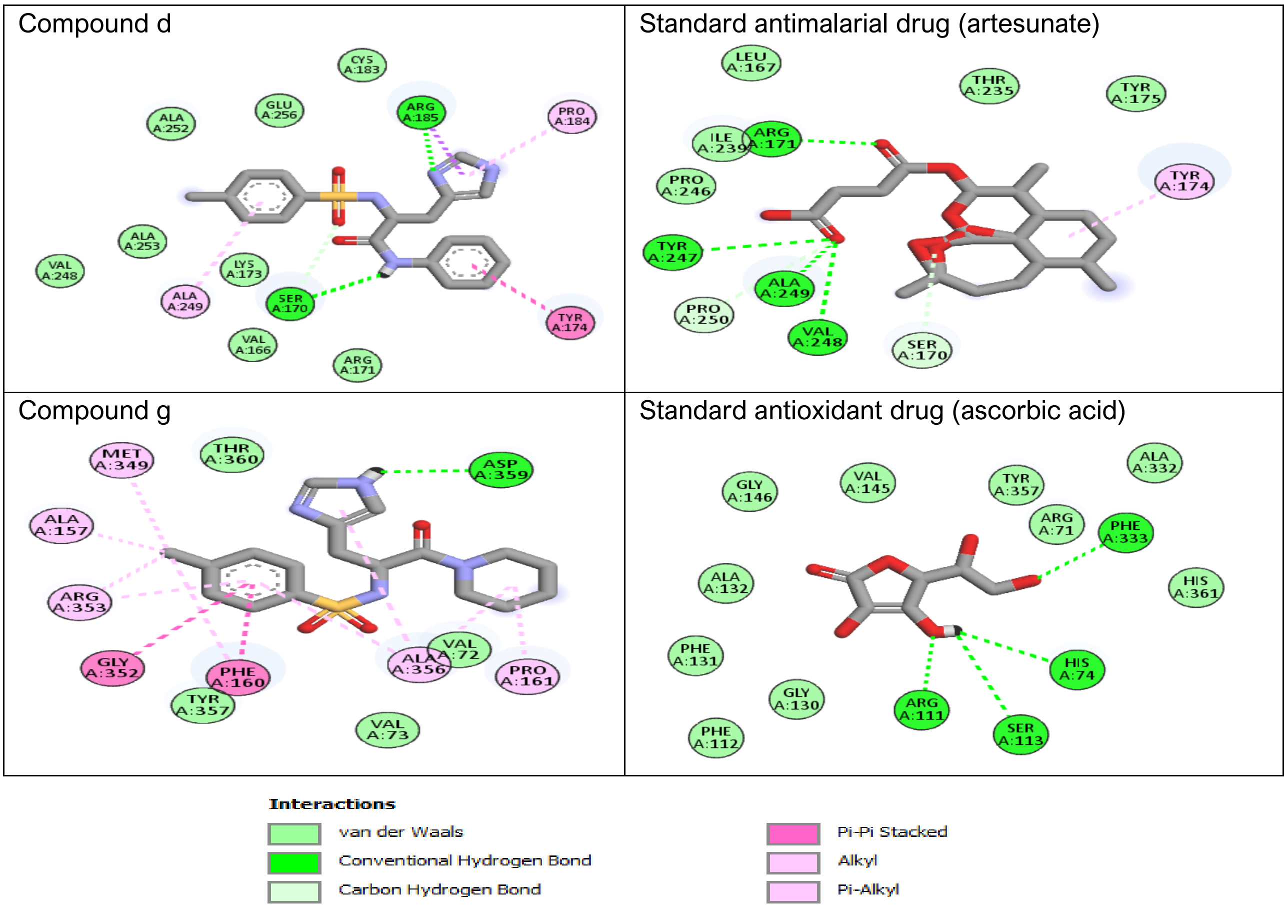


Figure 1: Surface interaction of compounds against *Lactate Dehydrogenase* for antimalarial and *Catalases* for antioxidant

3.3. *in vitro* antimalarial and antioxidant activity evaluation

Table 2: *Lactate Dehydrogenase* inhibitory activity*

Table 2, reveals the Lactate Dehydrogenase inhibitory studies result of the synthesized compounds and standard antimalarial drug with their various absorbance and percentage inhibition potential respectively.

Compound	Absorbance	Percentage (%) inhibition
d	0.301	77.12
e	0.565	57.07
f	0.437	66.79
g	0.279	78.80
h	0.544	58.66
ART	0.318	75.83

The standard antimalarial drug used is artesunate*

Table 3: DPPH radical scavenging inhibitory activity*

Table 3, depicts the DPPH radical scavenging inhibitory studies result of the synthesized compounds and standard antioxidant drug with their various absorbance and percentage inhibition potential respectively.

Compounds	Absorbance	Percentage (%) inhibition
d	0.259	73.38
e	0.380	60.95
f	0.322	66.91
g	0.202	79.23
h	0.316	67.52
AA	0.273	71.94

The standard antioxidant drug used is ascorbic acid*

3.2. Discussion

3.2.1. In silico antimalarial and antioxidant activities

Table 1 displays the calculated binding energies of the compounds. Interestingly, all the synthesized compounds showed a strong binding affinity with all the drug receptors employed in this study. For the antimalarial activity evaluation, most of the compounds tested on the parasitic *Lactate Dehydrogenase* receptor exhibited good *in silico* antimalarial activity. However, compared to artesunate (-7.4 kcal/mol), compounds d and g with binding energy (-7.8 and -7.5 kcal/mol), demonstrated good binding affinity. On the other hand, for the antioxidant activity evaluation, all the compounds tested on oxidant *Catalases* receptor revealed all the synthesized compounds (d – h) with binding energy ranging from (-8.0 to -6.5 kcal/mol) as having better binding affinity in comparable with ascorbic acid (-6.5 kcal/mol). The synthesized compounds tend to possess the potential to function as antimalarial and antioxidant agents.

Figure 1 displayed the surface interactions of compounds against *Lactate Dehydrogenase* and *Catalases* respectively. For the interactions against Lactate Dehydrogenase, Compound d shows two (2) main conventional H-bonds (Ser-170 and Arg-185). Furthermore, residues Val-166, Arg-171, Lys-173, Cys-183, Val-248, Ala-252, Ala-253, and Glu-256 are situated close to the complex, suggesting the possibility of Van der Waals interactions as well as Pi-Alkyl interactions with Tyr-174, Pro-184, and Ala-249. Moreover, the standard, artesunate shows four (4) main conventional H-bond (Arg-171, Tyr-247, Val-248, and Ala-249) and possible Van der Waals interactions with Leu-167, Ser-170, Tyr-175, Thr-235, Ile-239, Pro-246, and Pro-250 respectively. Similarly, for the interactions against *Catalases,* compound g reveals an H-bond (Asp-359), and possible Van der Waals interactions (Val-72, Val-73, Tyr-357, and Thr-360) residues are located close to the complex. The standard (ascorbic acid) shows four (4) main conventional H-bonds (His-74, Phe-333, Arg-111, and Ser-113). Additionally, it reveals possible Van der Waals interactions with Arg-71, Phe-112, Gly-130, Phe-131, Ala-132, Val-145, Gly-147, Ala-332, Tyr-357, and His-361 respectively.

3.2.2. In vitro antimalarial activity evaluation: *Lactate Dehydrogenase* inhibitory activity

Table 2 showed that all the synthesized compounds (d – h) demonstrated significant antimalarial activity. However, compounds d and g displayed the most promising antimalarial activities, inhibiting the protozoan parasite's growth, plasmodium falciparum which was used as a test microorganism and hence being the most potent antimalarial agent.

3.2.3. In vitro antioxidant activity evaluation: DPPH Scavenging inhibitory activity

Table 3 shows that all of the produced compounds (d – h) possessed potent antioxidant activity, which will reduce the progression of oxidative stress. However, compounds d and g showed excellent antioxidant activities out of all the tested compounds. Surprisingly, compound g outperformed ascorbic acid in the antioxidant activity evaluation and hence possesses the potential to decrease DPPH inhibition due to its increased absorbance.

4. CONCLUSION

The synthesis of histidine-derived sulphonamides bearing carboxamide functionality, molecular docking and *in vitro* pharmacological properties were successful. Five sulphonamides bearing carboxamide functionality (d – h) were synthesized. Spectral analytical techniques have been used to determine the structures of the compounds. *In vitro,* antimalarial and antioxidant studies of the synthesized compounds indicated that compounds (d and g) exhibited remarkable antimalarial and antioxidant properties, and this was supported by molecular docking assessment where they showed good free binding affinity to the amino acid residues.

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Sodium Dodecyl Sulphate-Aided Washing of Heavy Metals from Contaminated Soils at Auto-Mechanic Sites in Urban Makurdi

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ABSTRACT

Three automobile mechanic sites in urban Makurdi with many years of operation contaminated with petroleum products were assessed for remediation using Sodium Dodecyl Sulphate (SDS) as the surfactant. The main aim of the study was to remove heavy metals (Cd, Cr, Cu, Ni, Pb, Zn). Since soil pH, soil type, cation exchange capacity (CEC), particle size, organic matter content all affect removal efficiency, these parameters were also checked. All soil types were sandy, low in organic content which makes it good for soil washing. The surfactant Critical Micelle Concentration (CMC) was determined using electrical conductivity measurement as 5.0 mM, thereafter the effect of surfactant pH, surfactant concentration, soil/SDS ratio, washing and agitation time on the removal efficiency were studied. The results showed that pH 3 gave 70-90 % removal, pH 5 gave 50-60 % removal, pH 11 gave 10-30 % removal for heavy metals while, the surfactant concentration studies showed that increase in concentration of the surfactant increases the concentration of the contaminants in solution with 80-90 % removals, soil/surfactant ratio was also studied a ratio of 1:50 was recorded as the minimum and the best when considering surfactant aided soil washing, contact time of soil and surfactant was also studied 120 mins gave the highest removal.

KEYWORDS: Soil contamination, Heavy metals, Sodium Dodecyl Sulfate (SDS), Surfactant, CMC.

1. INTRODUCTION

Soil contamination from industrial and mechanical activities is a critical environmental issue that significantly impacts human health, ecosystems, and soil productivity. The presence of man-made substances or wastes not originally found in nature alters the natural soil environment [1]. Automobile mechanic workshops, in particular, contribute to substantial contamination due to the improper disposal of spent engine oil, lubricants, and other hazardous materials [2]. In Nigeria and other developing countries, the lack of proper waste disposal regulations exacerbates the problem, as many automobile workshops indiscriminately dump waste into nearby soil and water bodies [3].

The contaminants of concern, primarily heavy metals (HMs) such as lead (Pb), cadmium (Cd), zinc (Zn) can persist in the environment, bioaccumulate in living organisms, and pose long-term risks to human health. Soil pollution from mechanic workshops is particularly challenging to address due to the complex mixture of hydrophobic organic contaminants and metal pollutants that typically co-exist in these environments [4].

This study focuses on the application of surfactant-enhanced soil washing (SESW) using Sodium Dodecyl Sulphate (SDS) as an effective method for remediating contaminated soils. The goal is to determine the optimal conditions for removing HMs from mechanic workshop soils in Makurdi, Nigeria, a region heavily impacted by mechanic activities.

A few studies has shown that surfactants are used in remediation of HMs contaminated soils at automobile mechanic workshops such as the study conducted in Mexico by Salinas *et al*.[5]. Surfactant enhanced soil washing for the remediation of sites contaminated with pesticides [6]. Using SDS and Brij 30 at six different concentrations, when surfactants were added, the pesticide extraction increased considerably even for the lowest surfactant concentration, using SDS, 2,4-D extraction increased from 7% using water alone to 48.7% using 5 g/L SDS from 5 to 10 g/L a slight improvement in pesticide extraction from 48.7 to 50.7%. Using Brij 30, the surfactant resulted in an improvement in pesticide extraction efficiency compared to water alone but with a lower amount compared to SDS which was explained by the differences in the nature of the polar head group of the surfactants and SDS was more affected by surfactant concentration than in using Brij 30. Agarry *et al*[7] also carried out soil washing remediation of soil artificially contaminated with Naphthalene using palm kernel oil, coconut oil and waste cooking oil as solubilisation agents as alternatives to conventional solvents and surfactants.

2. MATERIALS AND METHODS

2.1. Chemicals/Reagents and Apparatus

Sodium dodecyl sulfate (SDS) (Kermel, 99.5%w/v) deionised water, distilled water, nitric acid, acetic acid , ammonium hydroxide, ammonium acetate, sodium acetate, magnesium chloride, calcium chloride, hydrogen peroxide, hydrochloric acid, hexametaphosphate Volumetric flasks, ae Adam digital chemical balance (max 180 g), thermometer, OAKTON pH meter pH/CON 510 series, EC 215 Conductivity meter (HANNA Instruments), Hy-2 Speed Adjusting Multi-purpose Vibrator, Genlab oven, beakers, conical flasks, < 2mm mesh sieve, whatman filter paper, funnel, serviette, Atomic Absorption Spectrophotometer.

2.2. Study Area and Sample Collection

Soil samples were collected from three major automobile workshops in Makurdi: Kanshio, North Bank, and New Garage. Samples were taken in black polythene bags, labeled, air-dried, homogenized, and sieved using a 2 mm mesh sieve [8, 9].

2.3. Soil Characterization

Key physicochemical properties of the soil samples were analyzed, including pH, bulk density, organic matter content, cation exchange capacity, and textural analysis [10, 11,] Heavy metal concentrations (Cd, Cr, Cu, Ni, Pb, Zn) using atomic absorption spectrophotometry (AAS) [12].

2.4. Surfactant Preparation and Soil Washing Experiments

Sodium dodecyl sulfate (SDS) was used as the surfactant for soil washing. The critical micelle concentration (CMC) was determined through electrical conductivity measurements, identifying 5.0 mM as the CMC, the concentration at which SDS starts to form micelles and efficiently remove contaminants [13].

Soil washing experiments evaluated four key variables:

1. **Initial pH of the surfactant solution**: SDS solutions were prepared at various pH levels (3, 5, 7, 9, and 11), and the soil samples were agitated at 200 rpm for 2 hours. Heavy metals and TPHs were measured after washing [15].

2. **Initial SDS concentration**: SDS concentrations ranging from 5 to 10 mM were tested to assess their effectiveness in removing contaminants [15].

3. **Soil-to-surfactant ratio**: The amount of soil (2 g, 3 g, 4 g, 5 g, and 6 g) was varied while keeping the surfactant concentration constant to examine the impact on contaminant removal efficiency.

4. **Washing time**: Contact times of 10, 30, 60, 90, and 120 minutes were tested to determine the effect of washing time on the removal of HMs [16].

2.5. Quality Assurance and Data Analysis

All measurements were conducted in triplicate, and statistical analyses were performed to assess the significance of the experimental parameters. The results were analyzed using two-way analysis of variance (ANOVA) to determine the effect of the different variables on contaminant removal [17].

3. RESULTS AND DISCUSSION

The results from the physicochemical analysis on the soil samples are presented in Table 1. The study revealed that the soils collected from the automobile workshops were predominantly sandy with low organic matter content. The bulk densities (1.3 to 1.56 g/cm³) and high sand composition (74-82%) indicate that the soils possess a high permeability, promoting the leaching of contaminants, into the groundwater. The low clay and silt content and reduced cation exchange capacity (CEC) suggest that the soils are suitable for remediation through soil washing.

Table 1: Physicochemical Properties of the Study Soils

Property	Kanshio (KS)	North-Bank (NB)	New Garage (NG)
pH	7.30	7.34	7.22
Bulk density (g/cm^3)	1.56	1.30	1.49
Sand (%)	80.24	74.24	82.24
Clay (%)	15.76	15.76	13.76
Silt (%)	4.00	10.00	4.00
Organic carbon (%)	1.54	1.72	1.64
Organic matter (%)	2.66	2.97	2.84
Na (cmol kg^{-1})	0.26	0.36	0.30
K (cmol kg^{-1})	0.27	0.30	0.29
Mg (cmol kg^{-1})	3.20	3.60	3.30
Ca (cmol kg^{-1})	3.60	3.80	3.40
Ea (cmol kg^{-1})	0.88	0.80	0.92
CEC (cmol kg^{-1})	8.21	8.82	8.21
Cd (mg kg^{-1})	3001.5	4444.5	4815.5
Cr (mg kg^{-1})	1595	2601	3656
Cu (mg kg^{-1})	1756	1960.5	2305.5
Ni (mg kg^{-1})	945	1030	1757.5
Pb (mg kg^{-1})	3522.5	4945.5	5316.5
Zn (mg kg^{-1})	1027.5	1862.5	2937.5

3.2. Critical Micelle Concentration (CMC) of Sodium Dodecyl Sulphate

The conductivity method was employed to determine the CMC of SDS by reading off the point of intersection of conductivity vs sodium dodecyl sulphate concentration plots (Figure 1).

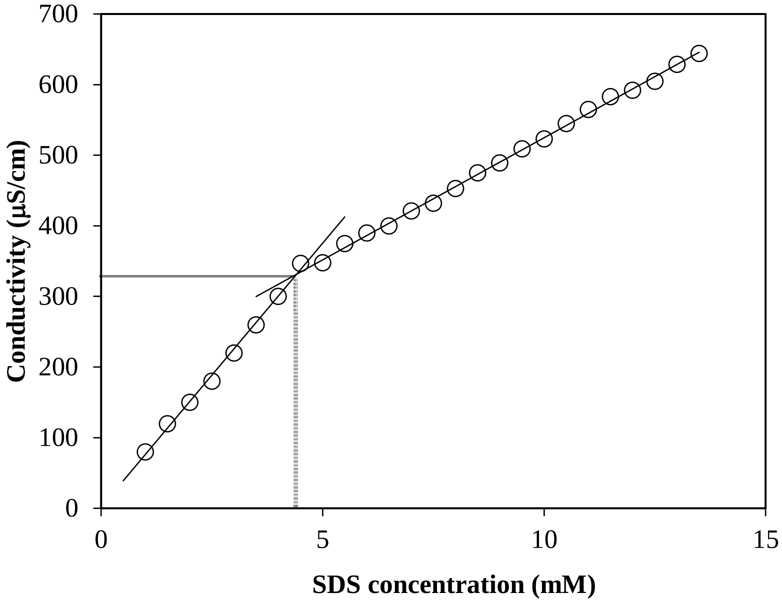


Figure 1: Plot of Conductivity vs Sodium Dodecyl Sulphate Concentration for Determination Critical Micelle Concentration (CMC)

The CMC of sodium dodecyl sulfate (SDS) was determined to be 5.0 mM. Below this concentration, surfactant molecules do not effectively reduce surface tension or mobilize contaminants, reinforcing the need for concentrations at or above this level for effective soil washing.

3.3. Effect of Initial SDS pH on heavy metals washing efficiency

The effect of initial surfactant pH on heavy metals removal efficiency from the contaminated soils was carried out, the actual metal concentration (mg/kg) leached from the soil by SDS are recorded in Table 2a-2c, the concentrations were transformed into percentage removal efficiencies with the aid of an equation similar to that reported by Wuana *et al* (2010) as:

$$\text{Percentage removal efficiency (\%)} = \frac{CmVm}{CsMs} \times 100$$

Where C_m is the concentration of metal in supernatant (mg/L), C_s is concentration of soil in mg/kg, V_m is the volume of supernatant in L, M_s is the mass of dry soil in kg.

The pH of the SDS solution was found to significantly impact the removal of heavy metals, with a pH of 3 showing the highest leaching efficiency for most metals, including cadmium (Cd), chromium (Cr), and lead (Pb). Soil from the New Garage site demonstrated the highest levels of heavy metal removal under acidic conditions, with Cd leaching reaching an average concentration of 3306 mg/kg. This finding aligns with previous studies indicating that acidic conditions enhance the desorption of heavy metals from soils.

Table 2a: Metal Concentration in mg/kg Leached by SDS from contaminated Soils at KS

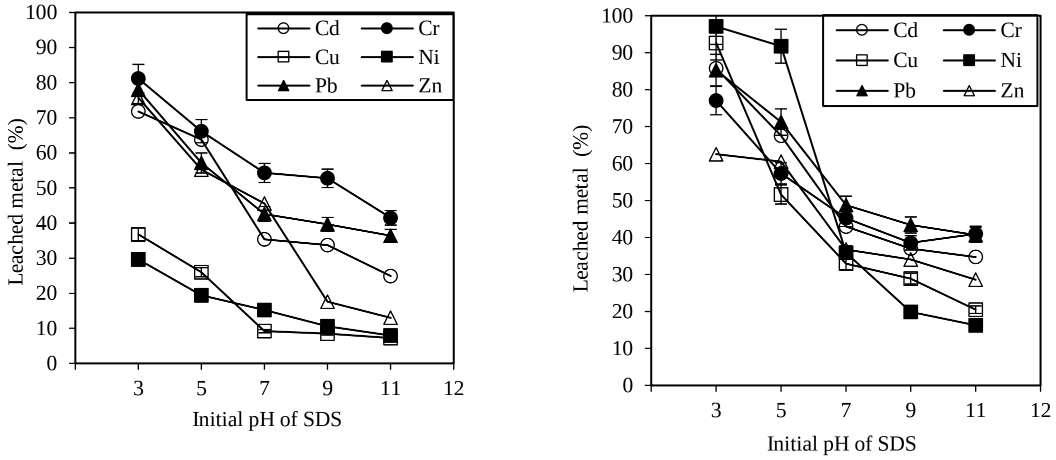
pH	Cd	Cr	Cu	Ni	Pb	Zn
3	2154.5	1294.5	645.5	280	2746.5	777.5
5	1915	1055.5	456	183.5	2011.5	567.5
7	1061.5	866	161	144	1499	468
9	1010.5	841	149.5	100	1396	180.5
11	748	662	127	75	1282	133.5

Table 2b: Metal Concentration in mg/kg Leached by SDS from contaminated Soils at NB

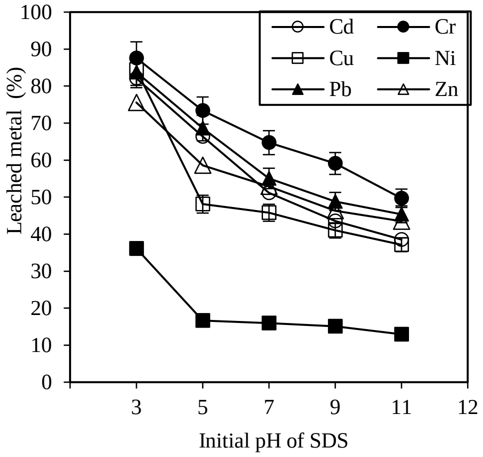
pH	Cd	Cr	Cu	Ni	Pb	Zn
3	3811.5	2004	1816.5	1000	4218	1165
5	3003	1491.5	1012.5	945	3522.5	1127.5
7	1911.5	1177	645.5	370	2411.5	685
9	1643	1003	566	205	2146	635
11	1543	1067	401.5	167.5	2011.5	532.5

Table 2c: Metal Concentration in mg/kg Leached by SDS from contaminated Soils at NG

pH	Cd	Cr	Cu	Ni	Pb	Zn
3	3952	3202	1946	635	4453	2220
5	3198	2683	1109	292.5	3650	1720
7	2461.5	2366	1055.5	280.5	2927	1552.5
9	2095.5	2160.5	945.5	265.5	2596	1360
11	1855.5	1816.5	856	227.5	2411.5	1277.5



Kanshio soil **North-Bank soil**



New Garage soil

Figure 2: Effect of Initial Sodium Dodecyl Sulphate pH on Heavy Metals Washing Efficiency in Contaminated Soils at Automobile Mechanic Sites

3.4 Effect of initial SDS concentration on heavy metals washing efficiency

The soil washing operations conducted using different surfactant concentrations in mg/kg are recorded in Table 3a-3c using the equation above and represented in figure 3a

Table 3a: Metal Concentration in mg/kg Leached by SDS from contaminated Soils at KS

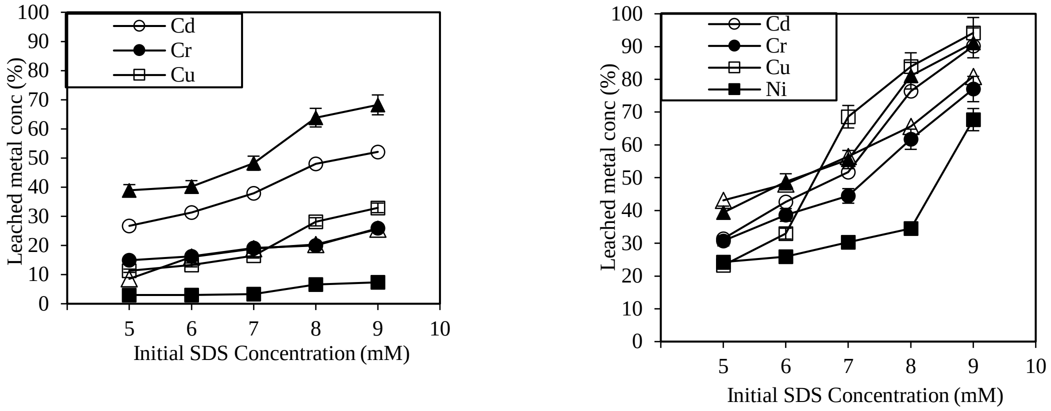
Conc.	Cd	Cr	Cu	Ni	Pb	Zn
5mM	1336.5	745.5	566	150	1946.5	427.5
6mM	1565	811	666	150	2011.5	82.5
7mM	1895.5	955.5	826	165	2411.5	945
8mM	2400.5	1000.5	1405.5	330	3192	1017
10mM	2605.5	1294.5	1645	367.5	3411.5	1280

Table 3b: Metal Concentration in mg/kg Leached by SDS from contaminated Soils at NB

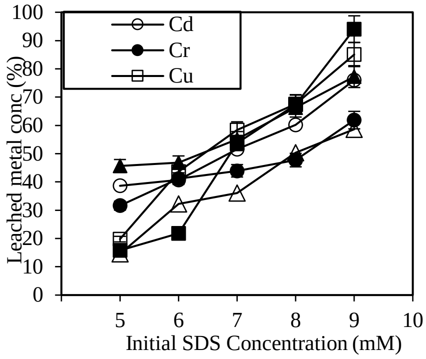
Conc.	Cd	Cr	Cu	Ni	Pb	Zn
5mM	1395.5	799.5	456	250	1946.5	802.5
6mM	1895	1005	645.5	266.5	2411.5	895
7mM	2295.5	1156	1345	3125	2746.5	1052.5
8mM	3395	1605.5	1645	355.5	4011.5	1222.5
10mM	4006	2004	1846	697.5	4507.5	1506

Table 3c: Metal Concentration in mg/kg Leached by SDS from contaminated Soils at NG

Conc.	Cd	Cr	Cu	Ni	Pb	Zn
5mM	1860.5	1156	456	277.5	2427	427.5
6mM	1961.5	1505.5	1006.5	384	2491	945
7mM	2485.5	1606.5	1345	945	2927	1061
8mM	2896	1745	1556	1883.5	3522.5	1480
10Mm	3661	2261.5	1960.5	1652.5	4107	1722.5



Kanshio **North Bank**



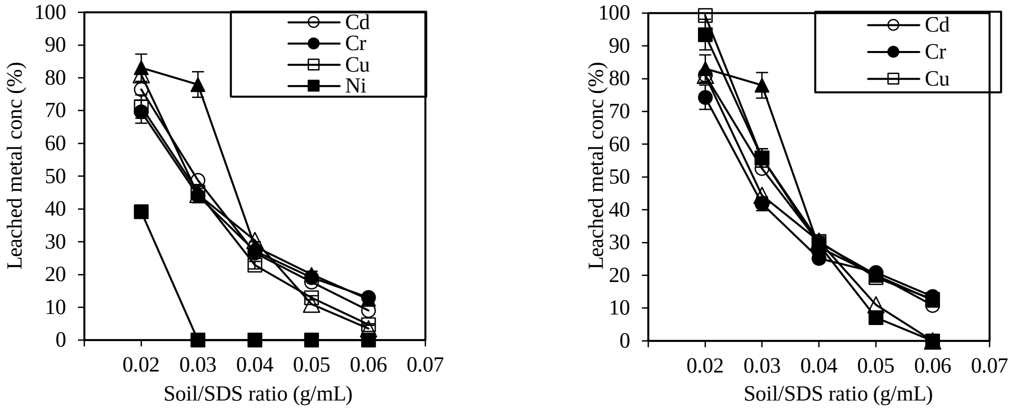
New Garage

Figure 3: Effect of Initial Sodium Dodecyl Sulphate Concentration on Heavy Metals Washing Efficiency in Contaminated Soils at Automobile Mechanic Sites

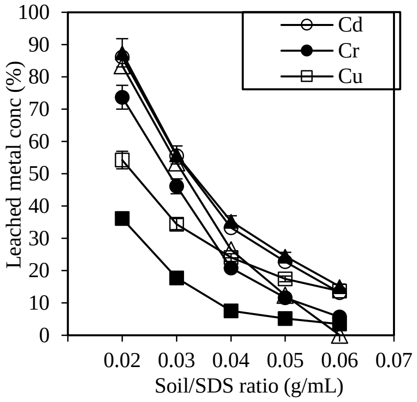
The research also highlighted the impact of SDS concentration on the efficiency of heavy metal removal. At a concentration of 10 mM, the highest removal efficiencies were observed for lead and cadmium for all sampling points as can be observed in figure 3a. These results suggest that higher concentrations of SDS are crucial for maximizing the removal of metals from contaminated soils.

3.5. Effect of soil/surfactant ratio on heavy metals washing from soil

Ratio of Soil/SDS conducted by varying the mass of soil at constant volume of SDS has metal concentration leached (mg/kg) and the percentage removal efficiency calculated also using the above equation is illustrated in figure 4.



Kanshio **North-Bank**



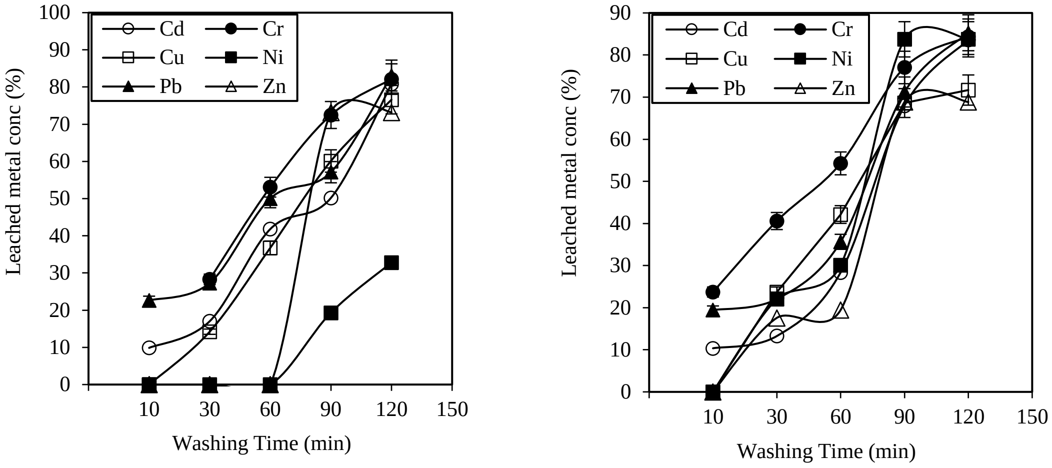
New Garage

Figure 4: Effect of Soil/Sodium Dodecyl Sulphate Ratio on Heavy Metals Removal from Contaminated Soils at Automobile Mechanic Sites

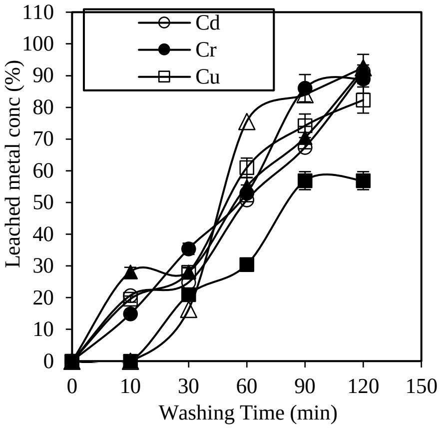
The soil-to-surfactant ratio was another key variable, with lower soil-to-surfactant ratios (0.02-0.03 g/mL) providing higher removal efficiencies for all metals, including cadmium and lead. A ratio of 0.02 g/mL yielded the highest removal efficiency, further supporting the efficacy of surfactant-enhanced soil washing at lower soil loads.

3.6 Effect of Washing Time on Heavy Metals Removal from Contaminated

Time an important factor influencing the removal of contaminants from soils in washing process by SDS at different contact times was also studied for HMs. The transformed concentrations to removal efficiencies in percentages are illustrated in figures5a for HMs.



Kanshio **North-Bank**



New Garage

Figure 5: Effect of Washing Time on Heavy Metals Removal by Sodium Dodecyl Sulphate from Contaminated Soils at Automobile Mechanic Sites

Washing time significantly affected the removal of heavy metals, with longer durations (120 minutes) yielding greater removal efficiencies for cadmium (87.91%) and lead (97.93%). The data suggest that extended washing time allows for more interaction between the surfactant and soil particles, improving contaminant desorption.

4. CONCLUSION

The remediation of soil contaminated by heavy metals is necessary so as to reduce associated human and plant risks and preserve the land for living and agricultural practices, the assessment of washing parameters for the SDS aided soil washing considered pH of surfactant, Concentration of surfactant, Soil/SDS ratio and Contact time of the surfactant and the contaminated soil, this was to assess the parameter that had the highest removal of the contaminant and the concentration of the surfactant at the greater removal, the greater removals were recorded for the high acid solution of the surfactant (pH 3) for heavy metals, higher concentration (for this research 10mM) above the CMC value had the highest removals, this implies that before a surfactant is used the CMC must be known, when the ratio of Soil/SDS was also checked it was discovered that a ratio of 1: 50 should be considered, the effect of washing time played a very significant role in washing contaminated soils, to achieve high percentage removal more time should be employed at least 3hours for heavy metals.

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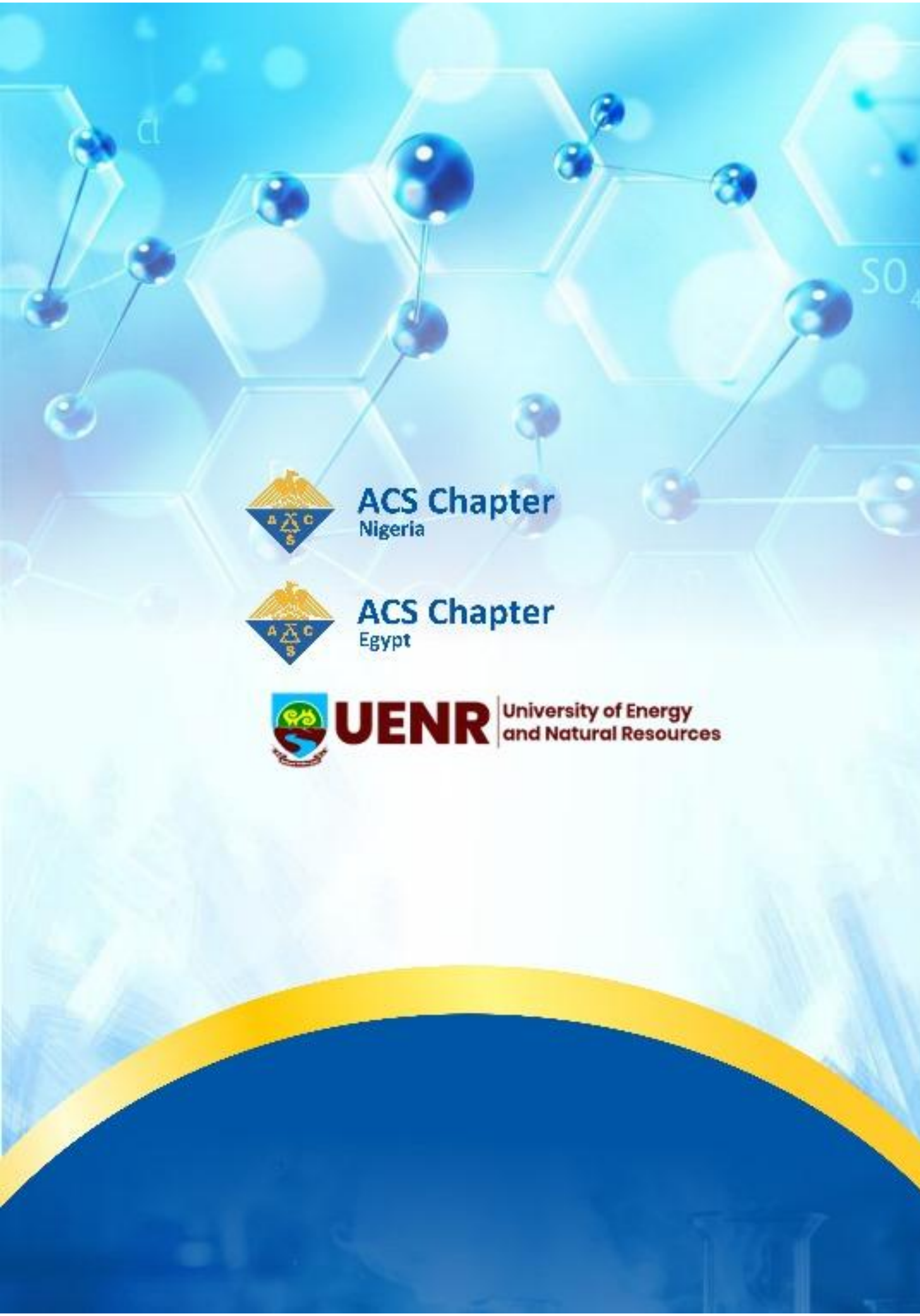

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