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Evaluation of the Cytotoxic and Antioxidant Extracts of *Artemisia Annua Whole Plant*

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ABSTRACT

Artemisia annua is an herb from Asteraceae family well known for its medicinal uses, particularly in the treatment of malaria. This study evaluates the cytotoxicity and antioxidant properties of *Artemisia annua* (sweet wormwood) extracts (n-hexane, ethyl acetate and methanol). The Brine Shrimp Lethality Test (BSLT) was carried out to access the toxicity of the extracts, and the antioxidant potential of various extracts was determined using DPPH assay, ABTS assay and Metal chelating activity assay (radical scavenging assay) compared with antioxidant controls BHA, ascorbic acid and α-tocopherol, respectively. The BSLT result showed high toxicity in all extracts, with the methanol extract showing the highest toxicity. The LC_{50} values (81.25 µg/L for n-hexane, 67.42 µg/L for ethyl acetate, and 51.59 µg/L for methanol) indicate that the lower the LC_{50}, the higher the potency of the extract. The radical scavenging assay exhibited great scavenging activity in all extracts, with the methanol extract outperforming the standards. These findings highlight the potential activity of *Artemisia annua* extracts for pharmaceutical applications, particularly in drug development, and also prove to be a potent source of biologically active compounds that can be further subjected to isolation of therapeutic antioxidant agents.

KEYWORDS: *Artemisia annua,* Cytotoxic, Antioxidant assay, drug discovery, natural products.

1. INTRODUCTION

Globally, medicinal plants have played a vital role in traditional healthcare systems and have continued to play a pivotal role in modern drug discovery. Historically, these plants are used for treating various ailments, often based on traditional knowledge and empirical experience.[1]

In recent times, the high cost of living and hike in healthcare services as well as drugs, have led to the increased use of medicinal plants by people, especially those in rural areas. Furthermore, several side effects have been reported from the use of conventional drugs globally, thus heightening the use of herbal remedies.[2]

Medicinal plants being the main sources of phytochemicals, contain bioactive compounds such as phenols, flavonoids, and alkaloids widely recognized for their potent antioxidant activities, which play an essential role in preventing oxidative stress-related diseases, such as cardiovascular disorders, neurodegenerative diseases and cancers through scavenging reactive oxygen species (ROS) and free radicals.[3,4] Thus, protecting cellular components from oxidative damage and protecting overall health.

Artemisia annua plant, known as sweet wormwood, has been used for treating fever and malaria.[5] Beyond its antimalarial properties, recent studies have highlighted the plant's rich phytochemical composition and its potential antioxidant and anti-inflammatory activities.[6]

These bioactive properties make the plant a potential therapeutic agent in cancer therapy and oxidative stress-related conditions.[7] Investigating the whole plant extract rather than isolated compounds allows for a synergistic evaluation of its therapeutic effects, potentially offering more potent and broadspectrum bioactivities.[6,7] This study aims to evaluate the cytotoxic and antioxidant properties of *Artemisia annua* whole plant.

2. MATERIALS AND METHODS

2.1 Plant collection and identification: The whole part of the plant was collected from ABU Zaria for five days, verified and authenticated (*Artemisia annua* ABU 0275) at the herbarium Botany section of the Department of Biological Science Ahmadu Bello University Zaria, Kaduna State, Nigeria.

2.2 Sample Treatment: The whole plant was washed to remove dirt, rinsed and air-dried, ground, and sieved. The powdered sample was weighed and stored in a clean polythene bags at room temperature.

2.3 Extraction: Cold extraction was done using the maceration method. A portion of (500g) of the powdered sample was soaked continuously with 2000ml of n-hexane, ethyl acetate, and methanol, respectively, allowed to stand for seven (7) days at room temperature, then decanted and filtered with 20cm filter paper. The filtrate was concentrated using a rotary evaporator to get the crude extract, while the residue was dried and soaked with ethyl acetate as well as methanol.

2.4 Collection: *Artemia salina* shrimp eggs were collected for cytotoxicity test from a standard laboratory abroad (cell- culture laboratory, United Kingdom) for BSLT.

2.5 Brine Shrimp Lethality Test (BSLT) Assays Procedure

Brine shrimp (Artemia salina) eggs were hatched in seawater for 48 hours to obtain larvae. Extracts (0.2 g) were dissolved in 2 ml solvent of extraction, and concentrations of 1000, 100, and 10 µg/ ml were prepared in triplicates with controls. Ten larvae were introduced into each vial, and survival was recorded after 24 hours. LC_{50} values were calculated using Finney probit analysis software. The extracts were labelled in the order n-hexane (001LN), ethyl acetate (002LN), and methanol (003LN).

2.6 Antioxidant Assay

Radical scavenging assay was done using DPPH assay, ABTS assay, and Metal chelating assay with the crude extracts, and the scavenging activity was expressed as percentage inhibition, using the standards BHA, ascorbic acid and α-tocopherol. The following concentration were done from 0.25, 0.5,1 and 1.5 mg/ml.[8]

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Cytotoxicity Results Using BSLT

LC50 values on n-hexane, ethyl acetate and methanol extracts are shown below as 81.27, 67.42 and 51.19, respectively, in Table 1. This indicates the toxicity of the extract against brine-shrimp larvae.

Table 1: Results of Brine-Shrimp Lethality Test (BSLT) of *A. annua* Whole Plant.

CRUDE EXTRACTS	SOLVENT OF EXTRACTION	LC_{50} (U/L)
001LN	n-Hexane	81.27 (139.06-455.34)
002LN	Ethyl Acetate	67.42 (42.16-518.18)
003LN	Methanol	51.59 (42.36-379.34)

LC_{50}< 1000µg/cm^3 = Toxic, LC_{50}>1000µg/cm^3 =Not Toxic *High/Low 95% Confidence Interval

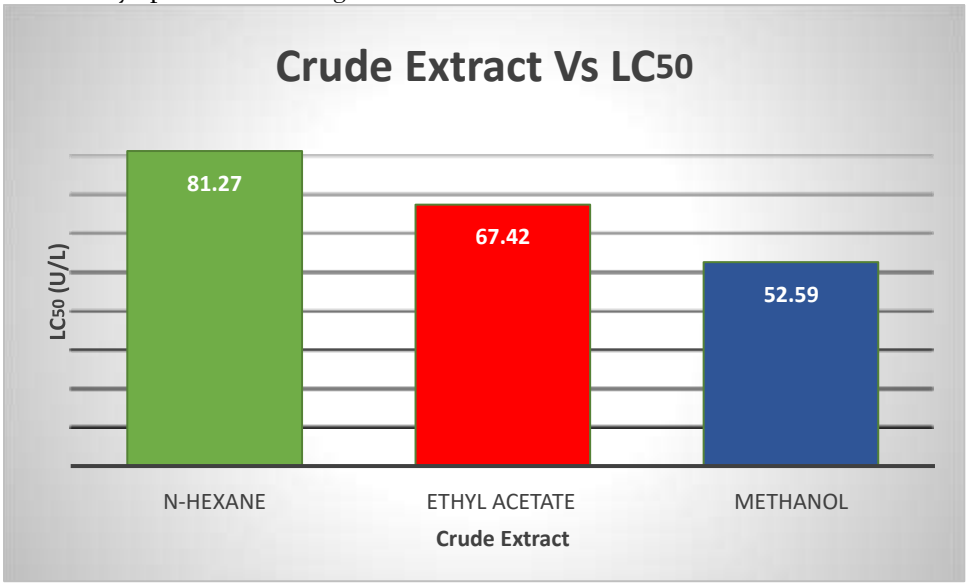

Figure 1: A bar Chart showing the LC_{50} of Artemisia annua whole plant extract.

3.2 Antioxidant Assay

These result shows the in-vitro lab-based tests used to evaluate the crude extract's ability to combat oxidative stress.

Table 2: Percentage (%) Inhibition of Crude Extracts and Standards against Assay type

This table shows the radical scavenging activity at the highest concentration, at 1.5 mg/ml

Extracts Type	Assay Type	Highest % @1.5mg/ml
Hexane	DPPH	73.3%
	ABTS	85.1%
	Metal Chelation	87.3%
Ethyl Acetate	DPPH	83.0%
	ABTS	851%
	Metal Chelation	87.3%
Methanol	DPPH	83.3%
	ABTS	89.1%
	Metal Chelation	88.3%
	Control	
Standard		Max % Inhibition (at 1.5 mg/mL)
BHA		81.2%
Ascorbic Acid		78.3%
α-Tocopherol		69.7%

DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) and Metal Chelation were used with the extracts.

Standards Used: BHA, Ascorbic Acid, α-Tocopherol.

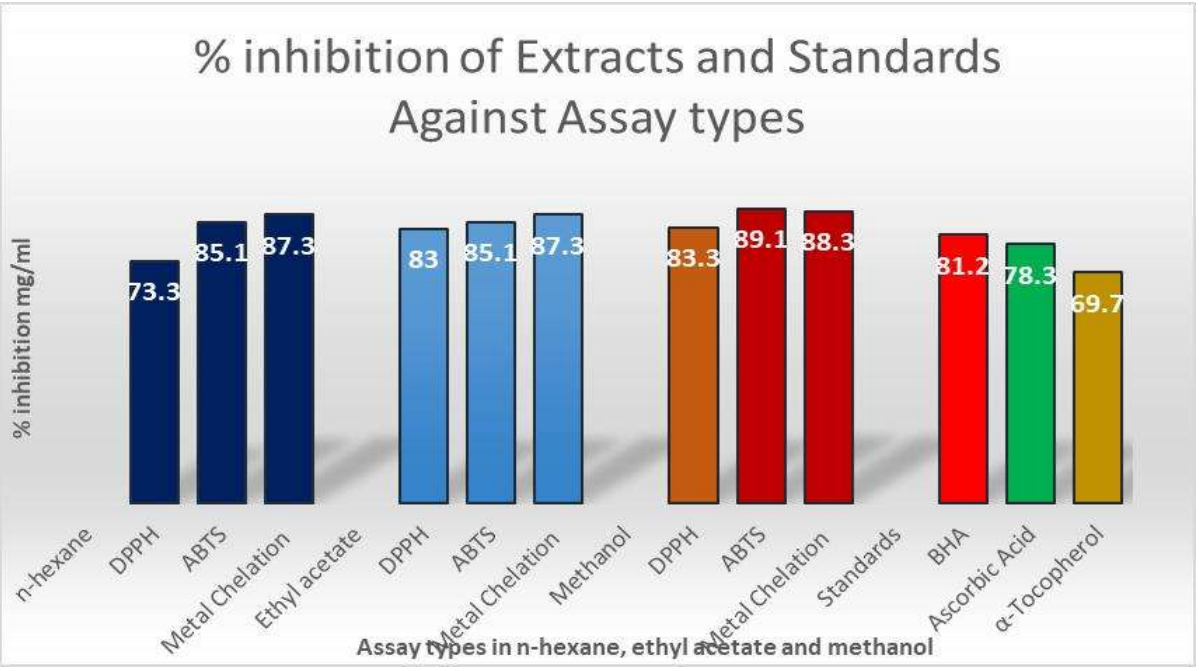

Figure 2: A bar chart showing the Percentage (%) Inhibition of Crude Extracts and Standard against Assay types.

3.2 Discussion

From figure 1, methanol extract has the lowest LC_{50} value (U/L) of (51.59), having the highest potency activity, followed by ethyl acetate extract (67.42) and n-hexane extract (81.27). indicating that methanol has the highest concentration of cytotoxicity bioactive compounds compared to n-hexane and ethyl acetate. No prior study has reported the cytotoxicity of *Artemisia annua* whole plant using Brine shrimp lethality test (BSLT).

From figure 2, the antioxidant assay using radical scavenging test shows that the extracts showed strong activity to compare the standards, with methanol extract exhibiting the highest scavenging activity in DPPH, ABTS and metal chelating scavenging assay followed by ethyl acetate and n-hexane. Thus, it can be said that methanol crude extract possesses good activity due to better extraction of polar antioxidants such as phenols and flavonoids being a polar solvent.[9]

4. CONCLUSION

This study evaluated the cytotoxic effects and antioxidant activity of three extracts from whole plant. These findings highlight methanol extract's superior activity compared to n-hexane and ethyl acetate extracts. This study confirms the cytotoxic potential of *A. annua* and suggest that the plant extracts, especially methanol and ethyl acetate fractions, possess strong antioxidant potential and may serve as an effective natural alternative to synthetic antioxidants. This study establishes a strong foundation for further investigation into isolation and characterization of active compounds from the crude extracts.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

REFERENCES

(1) Iqbal, S; Younas, U; Chan, K; Zia-Ul-Haq, M; Ismail, M. Chemical composition of *Artemisia annua L*. leaves and antioxidant potential of extracts as a function of extraction solvents. Molecules. 2012. 17(5), 6020-32.

(2) Ekor, M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacology. 2014, 4, 177.

(3) Mohamed, S; Mohamed, M. I. Antioxidants and Phytochemical Profiles in medicinal plants: Unravelling molecular mechanisms. Scholars international Journal of Biochemistry. 2024, 7(7), 106-117.

(4) Newman, D. J., Cragg, G. M. Natural products as sources of new drugs from 1981 to 2014. Journal of Natural Products. 2016, 79(3), 629–661.

(5) Septembre-Malaterre, A; Lalarizo Rakoto, M; Marodon, C; Bedoui, Y; Nakab, J; Simon, E; Hoarau, L; Savriama, S; Strasberg, D; Guiraud, P; Selambarom, J; Gasque, P. *Artemisia annua*, a Traditional Plant Brought to Light. International Jorunal Molecular Sciences. 2020, 21(14), 4986.

(6) Swamikannu, B; Umapathy, V. R; Natarajan, P. M; Nandini, M. S, Queency, S; Vimalarani, V; Rajinikanth, S. Unlocking the Therapeutic Benefits of Artemisia Annua: A Comprehensive Overview of its Medicinal Properties. Journal of Pharmacy and Bioallied Sciences. 2024, (5), S4248-S4253.

(7) Singh, V., Kaur, G. Unlocking the therapeutic benefits of *Artemisia annua*: A comprehensive overview of its medicinal properties. Journal of Pharmacy and Bioallied Sciences. 2024,16(5), 69–76.

(8) Nachiketa, B; Archna, S; Ashwani, K. Radical Scavenging and Antioxidant Potential of Nuts and Leaves Extracts of *Semecarpus anacardium (L.)*. American Journal of Plant Sciences. 2013, 4(8),1679-1683.

(9) Skowyra, M; Maria, G; Sergovia, F; Almajao, P. Antioxidant properties of *Artemisia annua* Extracts in Model food emulsions. Antioxidant (Basels). 2014, 3(1), 116-128.