Phytochemical Screening, Gas Chromatographic Analysis and Antimicrobial Efficacy of De-Fattened Methanolic Fresh Stem Extract of *Cissus arguta* Hook.f. (Sunset bell)

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ABSTRACT

Medicinal plants have been a fundamental part of human history for centuries, providing a vast array of therapeutic benefits. Investigating the antimicrobial potentials of *Cissus arguta* provides scientific support for its traditional use. This study focuses on conducting phytochemical screening, chromatographic analysis, and evaluating the antimicrobial efficacy of the fresh stem extracts of *Cissus arguta*. The methanol extraction of pulverized fresh plant stems was performed using soxhlet extraction, preceded by exhaustive de-fatting with n-hexane. Phytochemical analysis of the methanol extract identified eight compounds: alkaloids, glycosides, anthraquinones, flavonoids, saponins, steroids, tannins, and terpenoids. Chromatographic analysis revealed eleven components, primarily composed of esters, terpenes, and terpenoids (85.11%), followed by carboxylic acids (6.14%) and hydrocarbons (8.76%). The most abundant compound was 14-methyl-pentadecanoic acid methyl ester (29.90%), while the least abundant was the hydrocarbon 7-methyl-3,4-Octadiene (1.04%). The extract exhibited potent bactericidal effects against bacterial isolates with a minimum inhibitory concentration (MIC) ranging from 0.30±0.90 to 1.16±0.00 mg/mL across all tested microorganisms. These findings substantiate *C. arguta* as a promising broad-spectrum antimicrobial agent rich in bioactive phytochemicals, providing scientific validation for its traditional medicinal use.

KEYWORDS: *Cissus arguta, antimicrobial*, *GC-MS*, *phytochemicals*, *medicinal plants*.

1. INTRODUCTION

Throughout history, medicinal plants have been known to generate a vast range of chemicals that help them carryout critical biological tasks and defend themselves against predators.[1,21,25] *Cissus* comprises of nearly 400 species distributed globally,[2] representing the largest of all 14 *Vitaceae* genera. The genus of the plant has approximately 150 species in Africa, and 35 % of these species are found in West Africa. There are at least 40 species of the plant within the northern and southern Nigeria. The low seed production and propagation rate in all vegetation of *Cissus* species make its natural vegetation inconsequential.[3] In Nigerian traditional medicine, the stem is used in bone healing while the leaves when crushed, have been effectively used for treatment of microbial infections, inflammations, wounds, as well as cuts. It is also used for the treatment of intense fever, rheumatism, cough, arthritis, chest pain, gout, blockage of blood vessels, skin infections and wounds, body pains, dropsy, edema, febrifuges, as pain-killers, pulmonary troubles, and sexually transmitted infections, while the sap have been used for eye treatments and venereal diseases, as well as bone related diseases and disorders.[4,5] The phytochemicals including cardiac glycosides, flavonoids, alkaloids, tannins, terpenes, steroids, and saponins have been documented to be present in *Cissus spp*.[6] The plants are rich in various minerals and vitamins as well as other compounds deposited in the stems leaves, roots, and ash of the plant.[5] The study by Sudmoon *et al.*[7] identified δ-amyrin, δ-amyrone, friedelan-3-one, glycerin, and resveratrol, among others in *Cissus quadrangularis*. Similarly, GC-MS analysis of *Cissus vitiginea* revealed 3,7,11,15-Tetramethyl-2-hexadecen-1-ol and Tetradecanoic acid.[8] Sani *et al.*[9] examined the anti-inflammatory and analgesic effects of the root bark extract of *Cissus polyantha* and validated its traditional medicinal use. Edema *et al.*[4] focused on *Cissus arguta*, confirming its effectiveness in bone healing and antimicrobial activity against microbes involved in wound infections, supporting its therapeutic use in bone healing. This current study therefore seeks to determine some phytochemical constituents and antimicrobial potency of *Cissus arguta* with the aim of verifying its claims for use in medicine, thus increasing recorded species of plants with medicinal uses.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh plant samples of *Cissus arguta* were harvested in October 2023 from a vegetable garden at Ejeba area of Warri, Delta State, Nigeria. The plant was identified by Prof. B.Y. Abubakar of Botany Department, Ahmadu Bello University (ABU), Zaria, Nigeria, with voucher number **#**ABU0291. The leaves were plucked off the stems, and the stems washed under running tap water and then air dried in the laboratory for a period of 24 hours. The stems were then pulverized using a clean ceramic mortar and pestle, and weighed into a thimble. The weighted samples were then extracted successively in a soxhlet extractor using n-hexane, followed by methanol. The extract was concentrated and refrigerated (under 4 ^{o}C) until further use.

2.2 Phytochemical Analysis

The phytochemical studies of ten (10) different phytochemicals were conducted according to Sapunyo *et al.,*[10] with slight modification.

2.3 Gas Chromatography Mass Spectrophotometric (GC-MS) Analysis:

The GCMS analysis was conducted according to the procedures recorded by Smith and Brown[11] using Agilent 7809A with an HP-5MS column connected to a mass spectrometer. Helium was employed as the carrier gas for this study.

2.4 Microorganisms for Bioassay

Antimicrobial research utilized the methanolic extract of *Cissus arguta* to test its efficacy against four types of bacteria (*Bacillus subtilis* and *Staphylococcus aureus*, which are gram-positive; *Escherichia coli* and *Pseudomonas aeruginosa* which are gram-negative) and two types of medically significant fungi (*Candida albicans* and *Aspergillus niger*). Microbial slants were sourced from the Department of Microbiology, University of Benin, Nigeria, and were identified using standard procedures. Each microorganism was stored on Mueller Hinton agar in inclined tubes until use. Prior to testing, microbial cultures were diluted and standardized using the McFarland standard to achieve a uniform inoculum. Each test microbe was incubated in normal saline at 37 °C for 6 hours to standardize the inoculum concentration. The extraction, preparation, and microbial assays followed the method described by Hudzicki[12] with some modifications. The antimicrobial assays conducted include Zone of Inhibition for microbial susceptibility, minimum inhibitory concentration (MIC), and minimum bactericidal and minimum fungicidal concentrations (MBC/MFC). The MBC/MIC ratio for each microorganism was further determined from the respective MBC/MFC and MIC results. Generally, if the MBC/MIC ratio is less than or equal to 4, the agent is considered bactericidal, while a ratio greater than 4 suggests bacteriostatic activity.[13]

3. RESULTS AND DISCUSSION

3.1 Phytochemical Studies of methanol extracts of *C. arguta:*

The phytochemical studies of the methanol extract of *Cissus arguta* conducted in this study revealed the presence of steroids, alkaloids, glycosides, anthraquinones, flavonoids, saponins, tannins, and terpenoids, as shown in Table 1.

Table 1. Phytochemical Analysis of methanol extracts of *C. arguta*

Phytochemical	Test	Result
Tannins	Ferric Chloride	+
Saponins	Frothing	+
Alkaloids	-	+
Cardiac Glycosides	Keller-killan test	+
Carbohydrate	Benedict	-
Phlobotanins	-	-
Steroids	-	+
Flavonoids	-	+
Terpenes	Salkowsiac	+
Anthraquinones	Borntrager’s reaction for free anthraquinones	+

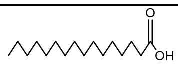
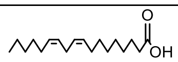
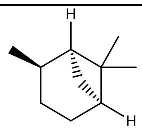
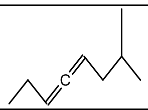
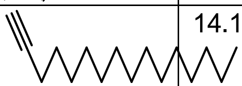
+ = *Present; - = Absent*

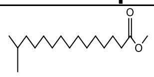
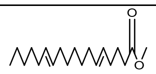
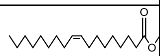
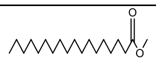
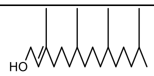
Alkaloids, phenolics, flavonoids, steroids, tannins, glycosides, saponins, and terpenes among others are distributed throughout various parts of plants.[14] The phytochemical analysis of the plant extracts (Table 1) in this study confirms the presence of these compounds, many of which are known for their biomedical properties. Terpenoids, for instance, have demonstrated diverse medicinal properties including antimicrobial, anticancer, anti-parasitic, antiallergic, antiviral, chemotherapeutic, anti-inflammatory, antihyperglycemic, and antispasmodic effects.[15,24,25] Similarly, tannins and saponins offer protection against pathogens in addition to their antimicrobial, anti-inflammatory, and antiulcer effects.[16,26,27] These findings underscore the potential of plant-derived phytochemicals in the development of therapeutic agents with various pharmacological benefits.

3.2 GC Analysis of methanol of *C. arguta:*

The chemical composition of *Cissus arguta* obtained from GC analysis is detailed in Table 2. The results as analyzed by GC (Gas Chromatography), reveals a diverse profile of compounds dominated by esters, terpenes, and terpenoids. Eleven compounds were identified, categorized as carboxylic acids (6.14 %), esters, terpenes, and terpenoids (85.11 %), and hydrocarbons (8.76 %).

Table 2. GC-MS Analysis of Methanol Extracts of *C. arguta*

Name of Compound	Structure	Retention time	Percentage Composition (%Area)
Carboxylic Acids			
n-Hexadecanoic acid		15.015	2.65
9,12-Octadecadienoic acid (Z,Z)-		16.686	3.49
	Total		**6.14**
Hydrocarbons			
2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.) Bicyclo[3.1.1]heptane		13.739	5.86
7-methyl- 3,4-Octadiene		13.993	1.04
1-Hexadecyne		14.180	1.86
	Total		**8.76**

Name of Compound	Structure	Retention time	Percentage Composition (%Area)
Esters, Terpenes and Terpenoids			
Pentadecanoic acid, 14-methyl-, methyl ester		14.631	29.90
Methyl 5,12-octadecadienoate		16.250	2.75
9-Octadecenoic acid (Z)-, methyl ester		16.313	21.85
9-Octadecenoic acid, methyl ester		16.370	9.17
Methyl stearate		16.541	14.45
Phytol		16.422	6.99
			85.11

Terpenes and terpenoids, in particular, have shown various antimicrobial, antioxidant, anti-inflammatory, and anticancer effects.[17,18] The high percentage (85.11%) of esters, terpenes, and terpenoids in *C. arguta* suggests potential medicinal benefits associated with these compounds. Although less abundant in percentage (6.14%), carboxylic acids play crucial roles in plant metabolism and can contribute to the overall chemical diversity and biological activities of plant extracts.[19,28,] Found at 8.76%, hydrocarbons are often present in plant extracts and can have various roles, including in plant defense mechanisms and as precursors for other bioactive compounds.[18] Further, studies have demonstrated that phytochemicals like terpenes and phenolic compounds, which are abundant in *Cissus* species, exhibit significant antimicrobial properties.[15,20] The composition of *C. arguta* suggests that it could serve as a potential source for natural antimicrobial agents, which is corroborated by the observed antimicrobial activity in the study. The active compounds identified likely contributed to the observed effects against microbes.[21,22,23] In summary, the chemical composition of *C. arguta,* characterized by its high bioactive contents, aligns with its reported antimicrobial properties and potential medicinal applications. This further highlights *C. arguta* as a promising candidate for pharmaceutical and therapeutic development.

3.3 Antimicrobial activity of methanol extracts of *C. arguta:*

Table 3(a) displays the Zones of Inhibition observed, while 3(b) gives the antimicrobial summary for various isolates at different concentrations of *C. arguta* extract.

Table 3. Antimicrobial Analysis of methanol extracts of *C. arguta*

Concentration of extract (mg/mL)	Zone of Inhibition* (mm) Gram negative bacteria		Gram negative bacteria		Fungi isolates	
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*A. Niger*	*C. albicans*
100	18.0± 0.0	18.0± 0.4	19.0±0.0	16.0± 0.0	14.0± 0.0	14.0± 0.4
50	16.0± 0.4	16.0± 0.4	17.0± 0.0	14.0± 0.4	11.0± 0.0	11.0± 0.0
25	14.0± 0.4	13.0± 0.0	15.0± 0.0	11.0± 0.0	--	--
12.5	14.0± 1.4	11.0± 0.0	12.0± 0.5	--	--	--
6.25	--	--	--	--	--	--
3.125	--	--	--	--	--	--
Negative control**	--	--	--	--	--	--
Positive control***	37.0± 0.4	38.0± 0.0	39.0± 0.5	37.0± 0.4	28.0± 0.0	28.0± 0.0
Zone of Inhibition at MIC* (mm)	14.0± 1.4	11.0± 0.0	12.0± 0.5	11.0± 0.0	11.0± 0.0	11.0± 0.0
MIC* (mg/mL)	1.23±0.02	1.23±0.00	1.24±0.01	1.19±0.00	ND$	ND$
MBC/MFC* (mg/mL)	0.34±0.00	0.31±0.23	0.30±0.90	0.33±0.15	1.16±0.00	1.16±0.00
MBC/MIC Ratio	3.50	3.97	3.98	3.71	ND$	ND$

**Inhibition zone diameter in millimeters around the well (diameter of the well, 9 mm, included); and Values are mean ± standard deviation of triplicate determinations **Negative control: distilled deionized water (for Bacteria and fungi); ***Positive Control: Gentamicin (10μg/ml) for bacteria, Tioconazole (30%) for fungi. $: ND= Not Determinable*

The methanol extract of *C. arguta* demonstrated significant antimicrobial activity against gram-positive (*B. subtilis*, *S. aureus*), gram-negative (*E. coli*, *P. aeruginosa*), and the yeast (*C. albicans*), particularly at higher concentrations (100 mg/mL). However, at 25 mg/mL, the extract no longer inhibited the growth of fungal species (*C. albicans* and *A. niger*) and at 12.5 mg/mL, it failed to inhibit *B. subtilis* and *S. typhi*. At concentrations of 6.25 mg/mL and below, the extract could not inhibit the growth of all tested microorganisms. The MBC/MIC ratios, although not determinable for fungi in this study, suggested bactericidal activity for the extract against bacteria, especially *S. aureus*, which showed the highest susceptibility across all concentrations tested. The study also referenced previous research[27,] highlighting the potential therapeutic applications of *Cissus arguta* in traditional medicine.

4. CONCLUSION

Phytochemical screening, chromatographic analysis, and the antimicrobial efficacy of methaolic extracts of fresh stem of *C. arguta* are presented in this study. The extract of *C. arguta* stem shows promises as a broad-spectrum antimicrobial agent, with its phytochemicals having potential effectiveness against both gram-positive and gram-negative bacteria, as well as certain fungi.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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