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Phytochemical Screening, GC Analysis and Antimicrobial Activity of N-Haxane Extract of Cissus arguta Hook.f. (Sunset bell)

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ABSTRACT

This study focused on determining the phytochemicals and the antimicrobial efficacy of the non-polar extract of the fresh stem of Cissus arguta using n-hexane. The phytochemical classes were determined by standard methods followed by Gas Chromatography – Mass Spectroscopic (GC-MS) analysis. Disc diffusion (Agar) method was used to determine the microbial sensitivity of extract against selected strains of micro-organisms, while broth dilution was used to determine the Minimum inhibitory concentration (MIC). The percentage yield of the extract was 1.03 %, revealing alkaloids, glycosides, saponins, steroids, tannins, and terpenoids via phytochemical studies. GC-MS results indicated free fatty acids as the major constituents (54.24 %) of the C. arguta n-hexane extract, closely followed by esters and terpenoids (41.50 %). The hydrocarbons were of alicyclic derivatives, with the least percentage composition (4.25 %). The extract showed antimicrobial potency against Escherichia coli (MIC:12.5 mg/mL; MBC: 25 mg/mL), Pseudomonas aeruginosa (MIC: 25; MBC: 25), Staphylococcus aureus (MIC: 25 mg/mL; MBC: 50 mg/mL), Bacillus subtlis (MIC:50 mg/mL; MBC: 100 mg/mL), Asperigillus niger (MIC: 25 mg/mL; MFC: 50 mg/mL), and Candida albicans (MIC:50 mg/mL; MFC: 100 mg/mL). The extract of C. arguta shows antimicrobial activity against all class of microbes, with the highest zones of inhibition against P. aeruginosa and S. aureus, but least active against the fungi strains. Specifically, C albicans show the highest resistance against the extract. The microbial activity and phytochemicals justify its folkloric usage.

1. INTRODUCTION

Plants are reliable source of medicine, and have been found to contain most biologically active phytochemicals (Ngo *et al.*, 2013) which are sometimes used in the manufacture

of newly synthesized drugs. These products, namely glycosides, carotenoids, steroids, tannins, flavonoids and terpenoids, are derived from various parts of plants' roots and shoots, suggesting the usefulness of almost all parts of plants (Ajibesin, 2011). Though these and many other biologically active phytochemicals are found in many

plants, the compounds are not well established due to limited knowledge of the chemistry and techniques in extracting them. Interestingly these vast range of chemical compounds were generated by plants to help them perform critical biological tasks and defend themselves against predators like insects, fungus, and herbivorous animals (Sandhya et al., 2006). There are still many different plant species that therapeutic compounds that have not yet been discovered, despite numerous exploration of the plant kingdom for pharmacological and phytochemical potentials (Trease and Evans, 1996). The main essence of phytochemical screening in addition to revealing the components of plant extracts, is finding compounds therapeutic bioactive for medications (Sheel et al., 2014).

Cissus are evergreen plants that can be perennials, shrubs, or climbers. occasionally have succulent stems or rootstocks, simple or palmately lobed leaves, and clusters of inconsequential flowers followed by tiny berries. The larvae of several *Lepidoptera* species, such as Hypercompeeridanus and Hypercompeicasia, consume Cissus species as food plants. Chimpanzees consume them as well (Rex and Ravi, 2020). Cissus arguta plant, classified under the Vitaceae family, is a crawling plant most seen in the swampy areas of the southern part of Nigeria, precisely the Niger Delta. It is an herbaceous climber with internodes and leaves attached to petiole, the leaves are typically dark green in colour. Its known as "Humbudi" in Ijaw language and "Oshimere" by the Itsekiris of Delta State.

Phytochemical research of *Cissus* plants have revealed several bioactive compounds, including alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins, and terpenes. Phytochemical studies reveal the existence of other compounds such as δ

amyrin, δ amyrone, friedelan-3-one, glycerin, isopentacosanoic acid, nonanol, pallidol, perthenocissine, phytol, phytosterols, piceatannol, resveratrol, taraxerol, and taraxeryl acetate (Sudmoon *et al.*, 2016). The root, leaves, stem, and ash of the *Cissus* plant contain high concentrations of mineral elements, vitamins, and other substances (Omotayo and Borokini, 2012).

Cissus arguta as previously investigated showed it has medicinal value having phytochemicals which have curative properties. Hence, further investigation of the phytochemical contents could be a panacea to novel drug discovery or the use of Cissus arguta to enhance orthodox treatment. The health benefits of Cissus arguta, are attributed to phytochemical constituents that have physiological actions on the human body. These compounds possess various antimicrobial and antibacterial activities. Cissus arguta locally is used for the treatment of certain diseases such as blockage of blood vessels, body pains, boils, bone related diseases and disorders, chest pain, cough, intense fever, inflammation of the lymphatic nodes, piles, peptic ulcers, rashes, skin and sexually transmitted infections, as well as wounds. This plant helps against muscle and bone pains, as well as gout, leucorrhoea, and tumours (Sarkar et al., 2016).

The screening of plant extracts has had an impressive history of identifying active agents. The choice of the stem part of *Cissus* arguta of interest in this work was based on its vast medicinal importance in West Africa. Therefore, there is reason for a scientific study to ascertain the medicinal potentials of this plant. The rising rate of mutation, especially in dangerous microorganisms, is one of today's biggest problems. With regard to these evolving strains, the majority of well-known, once reliable and efficient drugs appear to be losing their potency. This puts currently strain available on

chemotherapeutic agents (synthetic drugs) and asks for expanded and coordinated chemotherapy research. Drugs have historically been mostly sourced from medicinal plants. Utilizing a medicinal chemistry strategy, contemporary research initiatives to find and create new drugs are based on compounds obtained from plants.

The validation of the traditional usage of medicinal plants will ultimately provide scientific backing for the development of plants' bioactive constituents, which inturn, could provide novel compounds or precursors for the pharmaceutical industry. This study was therefore aimed at determining the phytochemicals, GC-MS profiling, and antimicrobial activity of *Cissus arguta* to verify its use in folkloric medicine.

2. MATERIALS AND METHODS

2.1 Extraction, Purification and isolation of Cissus arguta extract

Fresh stem of Cissus arguta samples were collected from Sapele Delta state. The plant was identified by Prof. B.Y. Abubakar of the Department of Botany, Faculty of Life sciences, Ahmadu Bello University, Zaria, Nigeria, with voucher number #ABU0291. Fresh samples of Cissus arguta were pulverized and extracted using soxhlet extractor. 50 g of sample was packed in a 500 mL soxhlet extractor. 300 mL of n-hexane was introduced to the extraction flask and was extracted for 3 hours. 20 g of anhydrous magnesium sulphate was then introduced to the flask containing extract in n-hexane and then swirled in order to remove any trace of water. This was then filtered, and the residue was washed thrice with n-hexane solvent into the flask containing the filtrate. The resulting filtrate was concentrated to a minimal volume using the Soxhlet extractor. The extract was poured into a weighed dish and allowed the rest of the hexane to evaporate and then weighed. Percentage yield of n-hexane extracts of *Cissus arguta* was then determined.

2.2 Phytochemical Analysis of Cissus arguta extract

The n-hexane extract obtained was screened for its photochemical constituents using standard procedures according to Sapunyo *et al.*, (2023). Phytochemical studies conducted include tannins (Ferric chloride test), saponins (Frothing test), alkaloids (Wagner's test), cardiac glycosides (*Keller- Killan test*), steroids, flavonoids, terpenes (Salkowsiac test), and anthraquinones (Borntrager's reaction for free anthraquinones).

2.3 Gas Chromatography Mass Spectroscopic Analysis of Cissus arguta extract

The bioactive chemical constituents of nhexane extract of fresh Cissus arguta was subjected to GC analysis using an Agilent 7809A GC connected to Agilent MS detector. The MS detector was equipped with split/splitless injector which is interfaced with a selective detector that operated at exactly 70 eV. The source temperature set at 200 °C upon an MS range of m/z 50-700 at 1428 amu/sec scan rate. The GC column model was HP-5MS with 30 m length, internal diameter 0.25 mm and a film thickness of 0.25 µm. The oven temperature programmed as follows: was initial temperature of 80 °C for 2 min, increased at 10 °C/min to a temperature of 240 °C for 6 mins. Helium was used as the carrier gas at a flow rate of 1 mL/min. Injection volume, linear velocity and pressure were adjusted at 1.0 µL, 362 cm/s and 56.2 KPa, respectively. The oven temperature was set at an initial temperature of 60 °C, and the final temperature of 280 °C with a gradual increase of 10 °C/min within 3 mins. Both the injector and detector temperatures were fixed at 250

°C. The relative percentages of the essential oil components were obtained by FID peakarea normalisation, all relative response factors being taken as 1.

2.4 Antimicrobial Analysis of Cissus arguta extract

Antimicrobial sensitivity was determined by the use of Kirby-Bauer disc diffusion method as reported by Hudzicki (2009) with some adjustments. Six (6) microbes consisting of two Gram-negative bacteria (that is, Escherichia coli (E. coli) and Pseudomonas aeruginosa (P. aeruginosa)) and two Grampositive bacteria (that is, Staphylococcus aureus (S. aureus) and Bacillus subtilis (B. subtilis)), with two fungi (that is, Candida albicans, and Aspergillus niger) were used in this study. All microbes were clinical isolates obtained from, and identified in the Department of Microbiology, University of Benin, Edo State, Nigeria.

Antimicrobial susceptibility Test: 15 mLs of freshly prepared Mueller Hinton agar was aseptically transferred into thirty-six (36) properly labelled sterile petri dishes (three plates per microorganism), and then allowed to cool and solidify. 0.1 mL of standard inoculum of each microbe was then transferred into, and evenly distributed on surfaces of sterilized medium contained in the plates. For each microorganism, three (3) petri dishes were used in order to have triplicate result for each organism. Seven (7) wells were then drilled on each plate, representing each concentration of the extracts as well as standard antibiotics as positive control (10 µg/mL gentamicin for bacteria, and 30% tioconazole for fungi) and Dimethyl sulfoxide (DMSO) as negative control. 0.1 mL of each concentration of the extracts of extracts and standards were then introduced respectively into separate wells on the medium, and incubated at 37 °C for 24 hours. Zone of inhibitions was then measured and the average of triplicate values calculated and recorded (in millimeters).

of minimum inhibitory Determination concentration: The minimum inhibitory concentration of the extract was determined using the broth double dilution method. 10 mLs of previously prepared Mueller Hinton broth was dispensed into test tubes. This was then sterilized at 121 °C for 15 mins, and then allowed to cool. The initial test solution of 10 mg/mL of extract was doubly diluted serially in sterile broth to achieve concentrations of 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, and 0.63 mg/mL. 0.1 mL of inoculum of each micro organism previously standardized with McFarland Turbidity Standard was then inoculated aseptically into all test tubes containing the different concentrations of extracts. This was followed by incubation at 37 °C for 24 hours. The lowest concentration of the extract in the sterile broth that shows no turbidity after incubation was noted as the minimum inhibitory concentration.

Determination of minimum bactericidal and minimum fungicidal (MBC)concentrations (MIC): To determine the minimum concentration that is growth inhibitor or lethal to test microbes, MBC and MFC were carried out against bacteria and fungi respectively. Freshly prepared Mueller Hinton agar was poured into sterile petri dishes and were allowed to solidify. The contents of the MIC (sterile broth that shows no turbidity after incubation) in the serial dilutions were then inoculated onto the prepared medium. Incubation was then made at 37 °C for 24 hours, after which the plates of the medium was observed for growth. Concentration of the extract in plates where no colony growth was observed after incubation was recorded as the MBC/MFC.

3. RESULTS

Table 1 presents the physical properties as well as the percentage yield of *C. arguta*. The

extraction of fresh stem of *C. arguta* using n-hexane yielded 1.03% of an amber colored extract.

Table 1: Percentage Yield and Appearance of n-hexane extract of C. arguta

Weight of Sample packed for Extraction (g)	50
Weight of extract (g)	0.515
Percentage yield (%)	1.03
Colour	Amber

Phytochemical analysis of n-hexane extract of *C. arguta* is represented in Table 2. Phytochemical screening of the n-hexane extract revealed alkaloids, glycosides,

saponins, steroids, tannins, and terpenoids, while flavonoids, anthraquinones and phlobotanins were absent.

Table 2: Phytochemical Screening of n-hexane extracts of *C. arguta*

Phytochemical	Test	Result	
Tannins	Ferric Chloride	+	
Saponins	Frothing Test	+	
Alkaloids	Wagner Test	+	
Cardiac Glycosides	Keller-Killan Test	+	
Steroids	Liebermann-Buchard Test	+	
Flavonoids	Alkaline Reagent Test	-	
Terpenes	Salkowsiac Test	+	
Anthraquinones	Borntrager's Reaction	-	

Key: + = present; - = absent

A total of fourteen (14) active components were determined from the GC-MS analysis containing three (3) carboxylic acids (54.24 %), seven (7) esters and terpenoids (41.50 %) and three (3) hydrocarbons (4.25 %). Tables 3, 4 and 5 shows the three class of compounds identified by the GC analysis.

Esters found in the extract were: 9-Octadecenoic acid (Z)-, methyl ester,

Pentadecanoic acid, 14-methyl-, methyl ester, Methyl stearate, 9-Octadecenoic acid (Z)-, methyl ester, Methyl 10-trans,12-cisoctadecadienoate, 8,11-Octadecadienoic acid, methyl ester, Undec-10-ynoic acid, undec-2-en-1-yl ester, 15-Octadecenoic acid, methyl ester. Free acids were oleic acid, n-hexadecanoic acid and cis-vaccenic acid. The hydrocarbons were cyclododecane, bicyclo [3.1.1] heptane, 2,6,6-trimethyl and octahydro- 4,7-Methano-1H-indene.

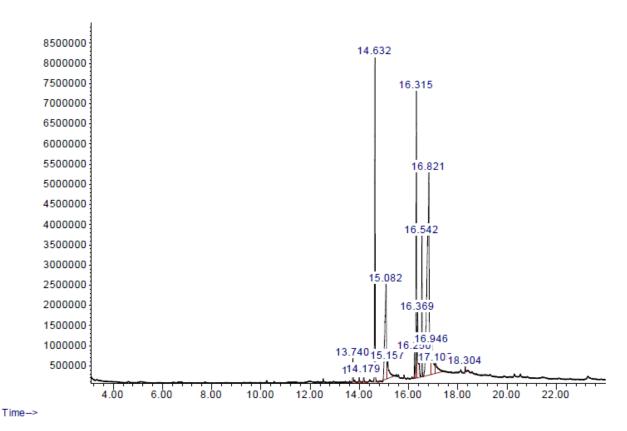


Figure 1: Gas Chromatogram of n-hexane Extracts of C. arguta

Table 3: Esters and terpenoids identified in the n-hexane extracts of C. arguta

Compounds	Structure	Retention time	Percentage composition (% area)
9-Octadecenoic acid (Z)-, methyl ester		16.313	18.32
Pentadecanoic acid, 14-methyl-, methyl ester		14.632	13.09
Methyl stearate		16.541	6.02
Methyl 10-trans,12-cis-octadecadienoate	VV-∕VVVV	17.107	2.29
8,11- Octadecadienoic acid, methyl ester		16.251	1.22

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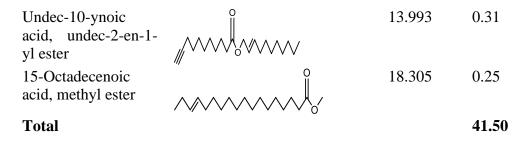


Table 4: Carboxylic acids identified in the n-hexane extracts of *C. arguta*

S/no	Compounds	Structure	Retention time	Percentage composition (% area)
1.	Oleic Acid	ОН ОН	16.821	37.05
2	n-Hexadecanoic acid	√ ОН	15.083	12.71
3	cis-Vaccenic acid		16.946	4.48
	Total	· · · · · · · · OH		54.24

Table 5: Hydrocarbons identified in the n-hexane extracts of *C. arguta*

s/no	Compounds	Structure	Retention time	Percentage composition (% area)
1	Cyclododecane		15.156	2.60
2	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl		13.739	1.19
3	Octahydro- 1H-indene 4,7-Methano-		14.180	0.46
	Total			4.25

Results for the antimicrobial analysis of n-hexane extracts of *C. arguta* are presented in Tables 6, 7 and 8. Accordingly, the n-hexane extracts of *C. arguta* shows antimicrobial activity against all class of microbes, with the

highest zones of inhibition against *P. aeruginosa* and *S. aureus*, but least active against the fungi strains. Specifically, *C albicans* show the highest resistance against the *C. arguta* extract.

Table 6: Zones of Inhibition of Different Isolates at Different Concentrations of n-hexane extracts of *C. arguta*

Concentration	on Inhibition* (mm)						
of extract	Gram negative bacteria		Gram negative	Gram negative bacteria		Fungi isolates	
(mg/ml)	E. coli	Pseudomonas	S. aureus	Bacillus	Asperigillus	Candida	
		aeruginosa		subtilis	niger	albicans	
12.5	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	3.0 ± 0.0	
25	0.0 ± 0.0	11.0 ± 0.1	0.0 ± 0.0	10.0 ± 0.3	2.0 ± 0.0	3.0 ± 0.0	
50	15.0 ± 0.4	17.0 ± 0.3	6.0 ± 0.4	14.0 ± 0.1	8.0 ± 0.3	9.0 ± 0.4	
100	16.0 ± 0.0	14.0 ± 0.1	11.0 ± 0.4	17.0 ± 0.1	8.0 ± 0.0	7.0 ± 0.0	
200	17.0 ± 0.1	18.0 ± 0.3	18.0 ± 0.1	15.0 ± 0.2	8.0 ± 0.2	7.0 ± 0.3	
Negative control**	0	0	0	0	0	0	
Positive control***	37.0	38.0	39.0	37.0	-	-	
Positive control***	-	-	-	-	28.0	28.0	

^{*}Values are mean \pm standard deviation of triplicate determinations **Negative control: DMSO (for Bacteria and fungi); ***Positive Control: Gentamicin (10 µg/mL) for bacteria, Tioconazole (30%) for fungi.

The Minimum Inhibitory Concentration (MIC) of n-hexane extracts of *C. arguta* are shown in Table 7. The MIC results revealed variation in the MICs of the extract for given micro-organisms. As observed, the extract exhibit appreciable MICs for bacteria (especially *E. coli*), but lower inhibitory

values for fungi. The lowest inhibition was 12.5 mg/mL (against *E. coli*). This was followed by an MIC of 25 mg/mL observed against *P. aeruginosa*, *S. aureus* and *A. niger*. Like *C. albicans*, *B. subtilis* gave the highest MIC values.

Table 7: Minimum Inhibitory concentration (MIC) of n-hexane extracts of *C. arguta*

Concentration of extract (mg/ml)	•		Gram negative bacteria		Fungi isolates	
	E. coli	Pseudomonas aeruginosa	S. aureus	Bacillus subtilis	Asperigillus niger	Candida albicans

0 (D)	MSO	-	-	-	-	-
control)						
100	-	-	-	-	-	-
50.0	-	-	-	-	-	-
25.0	-	-	-	+	-	+
12.5	-	+	+	+	+	+
6.25	+	+	+	+	+	+

Key: + = *Presence of Turbidity*; - = *Absence of Turbidity*

The Minimum Bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of n-hexane extracts of *C. arguta* are shown in Table 8. Again, the extract shows the least MFC against *C. albicans* and *B. subtilis*, with an MFC and

MBC values respectively of 100 mg/mL. This is followed by *S. aureus* and *A. niger*, having 50 mg/mL MBC and MFC respectively. The extract was most potent against *E. coli*, and *P. aeruginosa*, with an MBC of 25 mg/mL.

Table 8: Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of n-hexane extracts of *C. arguta*

Concentration of extract (mg/ml)	•		ve Gram negative bacteria		Fungi isolates	
	E. coli	Pseudomonas aeruginosa	S. aureus	Bacillus subtilis	Asperigillus niger	Candida albicans
0 (DMSO-control)	-	-	-	-	-	-
100	-	-	-	-	-	-
50.0	-	-	-	+	-	+
25.0	-	-	+	+	+	+
12.5	+	+	+	+	+	+

 $Key: + = Presence \ of \ Turbidity; - = Absence \ of \ Turbidity$

4. DISCUSSION OF RESULTS

Percentage Yield

Extraction from plants is empirical since various solvents are utilized at different conditions such as duration, method of extraction and temperature (Krishnananda *et al.*, 2017), and the percentage yield of extract is a function of the nature of plant and solvent, temperature of extraction, and extraction methods. The percentage yield of the n-hexane extract of fresh *C. arguta* was

1.03 %, a far cry from the yield of C. arguta from recorded literature, which ranged from 4 to 15 % (Kumar and Patel, 2021). The observed low yield may be as a result of the nature of plant materials. Dried plant materials often have higher concentration of extractable compounds because moisture can dilute the active ingredients (Santos and Silva, 2018). Further, presence of moisture may interact with the hydrophilic components of the plant, making the matrix non-penetrable by the hydrophilic n-hexane.

Phytochemical Analysis of n-hexane extracts of C. arguta

Phytochemicals studies of medicinal plants shown the presence of these compounds, most of which have been linked to anti-microbial, and other biomedical properties. Terpenoids have exhibited several medicinal properties like antimicrobial, antiparasitic, antiviral, ant allergic, chemotherapeutic, inflammatory, hyperglycemic and antispasmodic properties (Prakash and Gupta, 2009). Similarly, tannins and saponins have been reported to have exhibited protections against pathogens in addition to their antimicrobial, antiinflammatory and antiulcer effects (Mert-Turk (2006). A review by Srivastava, et al., (2020), discussed the antimicrobial effects of alkaloids, highlighting their potential as natural antimicrobial agents. Alkaloids extracted from plants have been found to possess antimicrobial properties, inhibiting the growth of certain microorganisms (Kumar et al., 2018). Specifically, alkaloids isolated from Berberis aristata showed remarkable antimicrobial potency against S. aureus, E. coli, and C. albicans (Singh et al., antimicrobial The effects of 2019). glycosides, as reviewed by Sharma et al., (2020), highlighted their potential as natural antimicrobial agents. Glycosides extracted from Vitis vinifera (grape) antibacterial potency against S. aureus, E. coli, and antifungal potency against C. albicans. (Kim et al., 2018). Similarly, glycosides isolated from Terminalia chebula exhibited antimicrobial effects against certain bacteria and fungi (Dey et al., 2019). Steroids have proven to be natural and synthetic antimicrobial agents (Patel et al., 2020). Natural steroids, such as cholesterol ergosterol, showed remarkable and antimicrobial potency against S. aureus and C. albicans (Liu et al., 2018). Further, synthetic steroids, such as prednisolone and dexamethasone, exhibited antimicrobial effects against certain bacteria and fungi (Rajput *et al.*, 2019).

GC Analysis of n-hexane extracts of C. arguta

Presence of these phytochemicals in the extracts validates their usage for therapeutic purposes. Jain *et al.*, (2010) had reported the presence of heptacosane, tentatriacontane and hexatriacontane from *Cissus quadrangularis*.

Antimicrobial analysis of n-hexane extracts of C. arguta

The antimicrobial results obtained in this study is consistent with those in literature where Cissus species have been found to exhibit antimicrobial effects against various microorganisms, including bacteria, fungi, and viruses. Dickson et al., (2012) had reported antibacterial and antifungal activity against Aspergillus flarus, B. subtilis, C. albicans, Enterobacter spp, Enterococcus feacalis, E. coli, Klebsiella pneumonia, Microsporuim camb, Proteus mirabilis, S. aureus, Streptococcus pneumonia, Tenea pedis as well as Trichophyton. Activity of C. arguta against A. niger as well and P. aeruginosa have not been previously reported. C. quadrangularis was reported to have exhibited remarkable antimicrobial activity against S. aureus, E. coli, and C. albicans (Jain et al., 2018), Cissus sicyoides according to de Souza et al., (2019), antimicrobial effects against exhibited certain bacteria and fungi, including methicillin-resistant S. aureus (MRSA). Similarly, the antimicrobial effects of various Cissus species, was discussed in a review by Ojewole et al., (2020), highlighting their potential as natural antimicrobial agents. The antimicrobial efficacy of Cissus arguta, as revealed in this study, may not be unconnected to the phytochemicals present in them. Results from this study reveal the presence of alkaloids, glycosides

steroids, among other phytochemicals. These plant chemicals have been found to exhibit antimicrobial effects against various microorganisms, including bacteria, fungi, and viruses.

5. CONCLUSION AND RECOMMENDATION

Phytochemical screening of the n-hexane extract of C. arguta revealed various bioactive metabolites which have shown antiparasitic, antimicrobial. antiviral. antiallergic, anti-inflammatory, chemotherapeutic, antihyperglycemic, antispasmodic and antiulcer effects. Presence of these compounds were confirmed by Gas chromatography (GC) analysis, and presence of these components in the extracts validated the usage of C. arguta for therapeutic purposes. The extract exhibited significant antimicrobial activity against a range of Gram-positive and Gram-negative bacterial strains, as well as some fungal strains. Overall, these findings suggest that the nhexane extract of C. arguta has potential therapeutic properties that can be explored for the development of novel drugs or natural remedies. However, further studies are needed to determine the safety, efficacy, and mode of action of the extract.

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COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this manuscript.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception, preparation, and design. Material preparation, data collection and analysis were performed by Mary Elire Edema and Hamzah Audu Bawa. Statistical analysis was performed by all authors. The first draft of the manuscript was written by Hamzah Audu Bawa and all authors commented on previous versions of the manuscript. The authors read and approved the final manuscript.

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