

COMPARATIVE ANTIMICROBIAL ACTIVITY AND BRINE SHRIMP CYTOTOXICITY OF TWO *CISSUS* SPECIES: *CISSUS ARGUTA* HOOK.F. AND *CISSUS QUADRANGULARIS* L. (VITACEAE)

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ABSTRACT

Antimicrobial resistance is one of the biggest challenges facing global public health. Most microorganisms have developed resistance to available conventional antimicrobial agents, hence, there is a need to develop new antimicrobials to combat this threat to public health. This study was conducted to investigate and compare the antimicrobial activity and brine shrimp cytotoxicity of *Cissus arguta* and *Cissus quadrangularis* leaves and the stems methanol extracts.

The plants (leaf and stem) were collected, air-dried, pulverized, and extracted with methanol (100%) concentrated *in vacuo*, and evaporated to dryness at a room temperature of 25 °C. The antimicrobial activity of the plant extracts against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Candida albicans*, and *Trichophyton rubrum* was investigated using the *in vitro* broth micro-dilution method with ciprofloxacin and ketoconazole as the reference antibacterial and antifungal drugs respectively.

The preliminary phytochemical screening showed the presence of saponin, flavonoid, tannins, alkaloids, anthraquinones, and steroids. The methanol extracts of *Cissus arguta* and *Cissus quadrangularis* demonstrated a higher antimicrobial activity against the gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and gram-negative (*Klebsiella pneumoniae* and *Escherichia coli*) bacteria than to the pathogenic fungi (*Candida albicans* and *Trichophyton rubrum*). Of all the extracts, *Cissus quadrangularis* stem extract exhibited the highest antimicrobial activity against both *Klebsiella pneumoniae* and *Escherichia coli* with MIC and MBC values of 12.5 µg/mL and 0.78 µg/mL respectively. It was also found to be the most potent against brine shrimps with LC₅₀ value of 10.62 µg/mL. This study suggests that *Cissus arguta* and *Cissus quadrangularis* possess antimicrobial and cytotoxic activities. In addition, the enhanced antimicrobial activity and higher brine shrimp cytotoxicity observed in the stem extract of *Cissus quadrangularis* suggest that it could serve as a potential source of more effective antimicrobial and cytotoxic agents.

Keywords: Antimicrobial activity, Cytotoxic Activity, *Cissus arguta*, *Cissus quadrangularis*

INTRODUCTION

Microscopic organisms are prevalent in nature and exist as unicellular, multicellular, or cell clusters [1]. Some are beneficial to life, while others are pathogenic and can cause serious harm. Microbial infections are part of the most debilitating illnesses that affect humans and animals. They are the major cause of morbidity and mortality among the world population, especially in developing countries [2]. Microbes are constantly growing resistance to conventional antimicrobials, thus increasing the threat posed by microbial infections [2]. The multi-resistance of microorganisms to available antimicrobial agents is associated with their genetic ability to acquire resistance mechanisms such as genes encoding enzymes like β lactamases, genes that alter the bacterial cell wall resulting in no binding site for the antimicrobial agent and efflux pumps through conjugation, translation, and transduction as well as transmit resistance to sensitive strains [3, 4]. The alarming spread of drug-resistant microorganisms and limited access to anti-infective drugs in some populations emphasizes the urgent need to discover and develop new antimicrobials to combat this epidemic, considered by some experts as “the post-antibiotic era” [5, 6]. Antimicrobials are naturally occurring or synthetic organic compounds, which at low concentrations can inhibit or destroy selective microorganisms [7, 8]. The discovery of drugs from medicinal plants has played an important role in the treatment of microbial infections. Studies on the effects of plant extracts against resistant microbes have shown that novel compounds from plants may not have the problem of antimicrobial resistance, some plant extracts can boost antimicrobial activity

or reverse antimicrobial resistance, and hence they have been classified as resistance modifiers [3]. Natural plant extracts are an important source of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties [3]. The presence of these secondary metabolites makes them suitable candidates among antimicrobial agents [9].

Genus *Cissus* belongs to the family Vitaceae, and consists of about 350 species, among which have been used globally in traditional medicine to treat several ailments such as infections, arthritis, obesity, cancer, and diabetes [10, 11, 12]. *Cissus* plants have been explored for their extensive range of biological activities (anti-microbial, anti-inflammatory, anti-tumor and anti-diabetic) which are attributed to certain bioactive components such as triterpenes, fatty acids, glycerolipids, steroids, phytols, cerebrosides, flavonoids and stilbenes [10]. *Cissus arguta* Hook.f. is one of the less explored species of the genus *Cissus*. It is an herbaceous climber found in the rainforest of southern Nigeria [13, 14]. Locally, it is extensively used in the treatment of certain infections such as intense fever, cough, chest pain, inflammation of the lymphatic nodes, blockage of blood vessels, rashes, boils, wounds, skin, and sexually transmitted infections, body pains, as well as bone-related diseases and disorders by herbalists [13,14]. Although the medicinal properties of various varieties of *Cissus*, such as *Cissus populnea*, *Cissus quadrangularis*, *Cissus palmata* and *Cissus alata* have been extensively studied, there seems to be little report on the medicinal potentials of *Cissus arguta* despite its uses. *Cissus arguta* contains phytochemicals such as tannins, flavonoids, alkaloids, glycosides, steroids, phenolic

compounds, eugenols, and saponins [13 14]. The plant has been described by Ogunmefun *et al.*, and Dickson *et al.*, [13, 14] for its anti-inflammatory and wound-healing activities. *Cissus quadrangularis*, on the other hand, is a succulent plant which is usually found in tropical and subtropical xeric wood [15]. It is fleshy, cactus in nature. Ethno-botanically, it is mostly used in treating female disorders (menopause, libido, and menstrual disorder) and treating bone disorders (increasing bone mass or accelerating fracture healing rates) which gives it the traditional name of the 'Bone Setter' [16, 17]. It is also used as a body-building supplement and in the treatment of obesity, gout, syphilis, venereal diseases, leucorrhea, worm infestation, anorexia, diabetes, peptic ulcer, hemorrhoids, and high cholesterol [16, 17]. In the Northeastern states of India, its stem is used as a vegetable [16]. Phytochemical studies of *Cissus quadrangularis* have shown the presence of various versatile constituents such as flavonoids, triterpenoids, Vitamin C, stilbene derivatives, alkaloids, alkaloids, tannins, saponins, and many others [16, 17, 18]. Pharmacological activities include bone fracture healing, analgesic, anti-ulcer, antimicrobial, anti-hemorrhoidal, gastro-protective, antioxidant, anti-

inflammatory, and stimulatory activities [16, 17, 18]. The study's main objectives were to evaluate and compare the antimicrobial activities and brine shrimp lethality of *Cissus arguta* and *Cissus quadrangularis* leaf and stem extracts.

RESULTS

Preliminary Phytochemical screening

The preliminary phytochemical screening indicated that saponins, tannins, flavonoids, steroids, and alkaloids were present in *Cissus arguta* leaf extract while anthraquinone was absent (Table 1). Saponins, flavonoids, and alkaloids were present in *Cissus arguta* stem extract while tannins, steroids, and anthraquinones were absent. Flavonoids, alkaloids, tannins, steroids, and anthraquinones were present in *Cissus quadrangularis* leaf extract while saponin was absent. Saponins, flavonoids, alkaloids, tannins, steroids, and anthraquinones were present in *Cissus quadrangularis* stem extract.

Table 1: Phytochemical Screening of *Cissus arguta* and *Cissus quadrangularis*

Test	<i>Cissus arguta</i>		<i>Cissus quadrangularis</i>	
	Leaf	Stem	Leaf	Stem
Saponins	+	++	-	++
Tannins	+	-	++	++
Flavonoids	++	+	++	++
Steroids	+	-	++	++
Alkaloids				
<i>Dragendorff</i>	++	+	++	++
<i>Mayer</i>	+	+	++	+
<i>Wagner</i>	+	+	++	++
Anthraquinones	-	-	+	++

Key: + = Present, ++ = Moderately Present, - = Absent

ANTIMICROBIAL ASSAY

The activities of the leaf and stem methanol extracts of *Cissus arguta* and *Cissus quadrangularis* tested on Gram-positive, Gram-negative bacteria, and Fungi are compared, the results are displayed in Figures 1-4. The minimum inhibitory concentration (MIC) is the least concentration of a plant extract that shows no growth of microbial isolates in broth, while the minimum bactericidal concentration (MBC) is the least concentration that will completely kill a particular microorganism. In this study, the MIC observed for *Cissus arguta* leaf extract was 25 µg/mL against *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli*, 50 µg/mL against *Bacillus subtilis* and *Trichophyton rubrum* (Fig. 1). On the other hand, the MBC was 50 µg/mL against *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia*

coli and *Candida albicans* and 100 µg/mL against *Bacillus subtilis* and *Trichophyton rubrum* (Fig. 2). In comparison with *Cissus quadrangularis* leaf extract, the MIC was observed to be 50 µg/mL against *Klebsiella pneumoniae*, *Bacillus subtilis* and *Trichophyton rubrum*, 100 µg/mL against *Escherichia coli*, and 200 µg/mL against *Staphylococcus aureus* (Fig. 1) while the MBC was observed to be 100 µg/mL against *Klebsiella pneumoniae*, *Bacillus subtilis* and *Trichophyton rubrum* (Fig. 2). *Cissus arguta* and *Cissus quadrangularis* leaf extracts could not inhibit the growth *Candida albicans* (no MIC was recorded). All microbial isolates were completely killed by *Cissus arguta* leaf extract while *Cissus quadrangularis* leaf extract could not completely kill *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*.

KEY: KP = *Klebsiella pneumoniae*, SA = *Staphylococcus aureus*, EC = *Escherichia coli*, BS = *Bacillus subtilis*, CA = *Candida albicans*, TR = *Trichophyton rubrum*, CAL = *Cissus arguta* leaf, CQL = *Cissus quadrangularis* leaf, CAS = *Cissus arguta* stem, CQS = *Cissus quadrangularis* stem

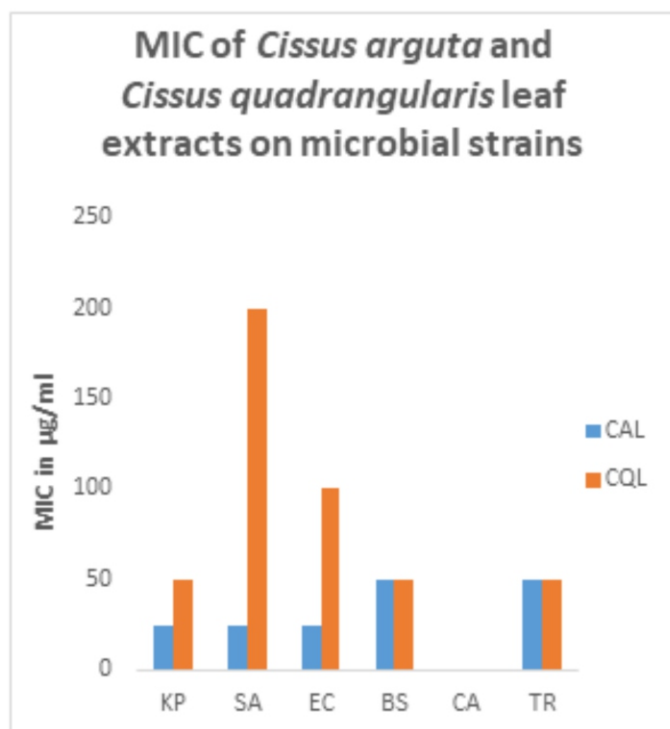


Fig 1: A chart comparing the MIC of crude methanol extracts of *Cissus arguta* and *Cissus quadrangularis* leaves on 6 microbial strains.

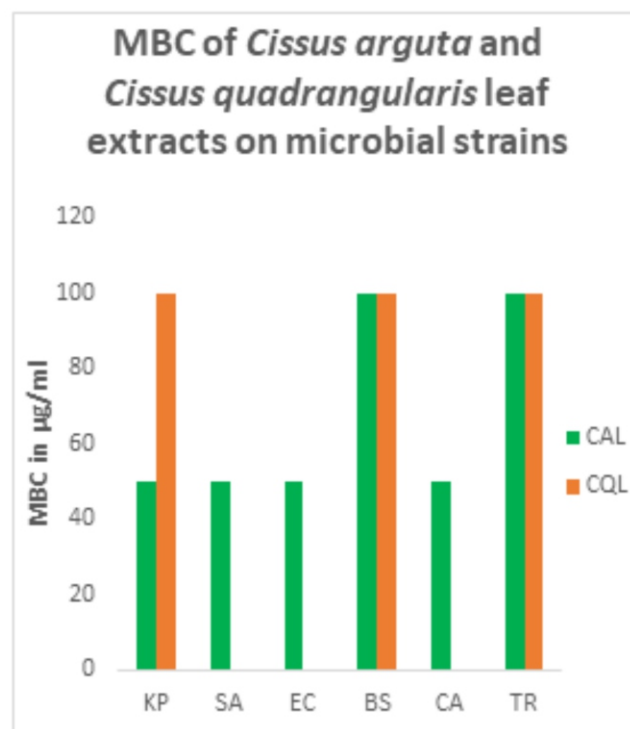


Fig 2: A chart comparing the MBC of crude methanol extracts of *Cissus arguta* and *Cissus quadrangularis* leaves on 6 microbial strains.

The MIC observed for *Cissus arguta* stem extract was 25 µg/mL against *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli*, 100 µg/mL against *Bacillus subtilis* and *Trichophyton rubrum* (Fig. 3) while the MBC was found to be 50 µg/mL for *Klebsiella pneumoniae*, *Escherichia coli* and *Candida albicans* and 200 µg/mL for *Bacillus subtilis* and *Trichophyton rubrum* (Fig. 4). Compared with *Cissus quadrangularis* stem extract, the MIC was 12.5 µg/mL against *Klebsiella pneumoniae*, 25 µg/mL against *Escherichia coli* and *Bacillus subtilis* (Fig. 3) and the MBC was 0.78 µg/mL against *Staphylococcus aureus*, 25 µg/mL for *Klebsiella pneumoniae*,

and 50 µg/mL for *Escherichia coli*, *Bacillus subtilis*, *Candida albicans*, and *Trichophyton rubrum* (Fig. 4). *Cissus arguta* stem inhibited the growth for all isolates except that of *Candida albicans* while *Cissus quadrangularis* stem extract inhibited the growth for all the isolates except *Staphylococcus aureus*, *Candida albicans*, and *Trichophyton rubrum*. All strains of microorganisms were completely killed by *Cissus quadrangularis* stem extract while only *Staphylococcus aureus* isolate could not be completely killed by *Cissus arguta* stem extract.

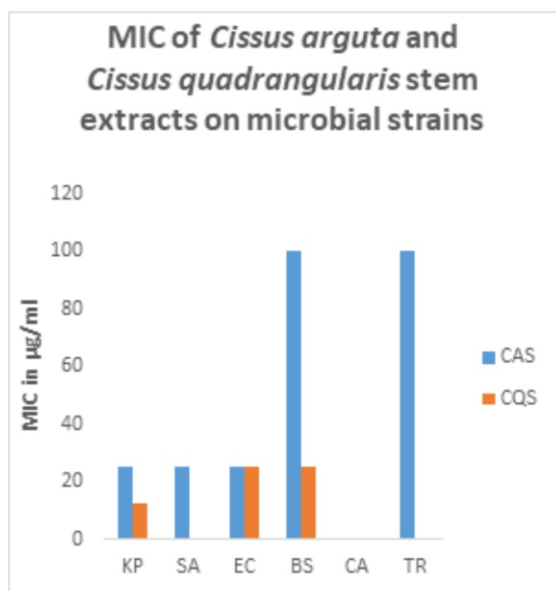


Fig 3: A chart comparing the MIC of crude methanol extracts of *Cissus arguta* and *Cissus quadrangularis* stems on 6 microbial strains.

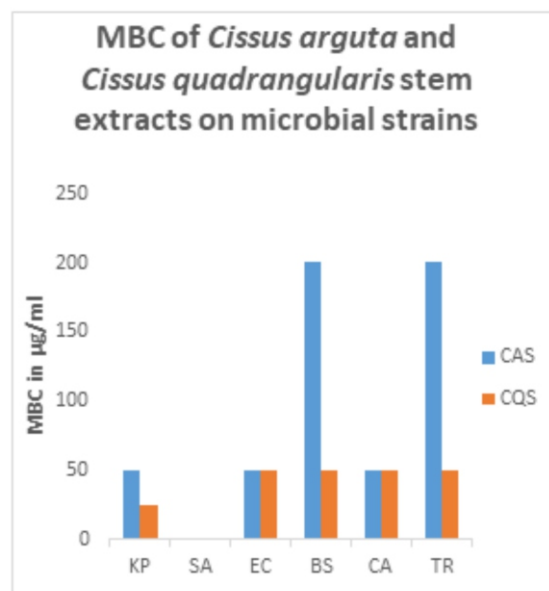


Fig 4: A chart comparing the MBC of crude methanol extracts of *Cissus arguta* and *Cissus quadrangularis* stems on 6 microbial strains.

KEY: KP = *Klebsiella pneumoniae*, SA = *Staphylococcus aureus*, EC = *Escherichia coli*, BS = *Bacillus subtilis*, CA = *Candida albicans*, TR = *Trichophyton rubrum*, CAL = *Cissus arguta* leaf, CQL = *Cissus quadrangularis* leaf, CAS = *Cissus arguta* stem, CQS = *Cissus quadrangularis* stem

The MIC and MBC values show that in general, antimicrobial activity of methanol extracts of the 2 *Cissus* species is much higher on pathogenic bacteria than fungi. The lowest and hence most effective MIC and MBC values were that of the methanol extract of *Cissus quadrangularis* stem against *Klebsiella pneumoniae* (MIC = 12.5 µg/mL) and *Staphylococcus aureus* (MBC = 0.78 µg/mL).

Overall, the methanol extract of *Cissus quadrangularis* stem showed the highest activity and is considered the most efficient against all tested microbial strains while the least activity was shown by the action of the leaf extract of *Cissus quadrangularis*. The MIC value of the positive control (Ciprofloxacin and Ketoconazole) against the tested bacteria and fungi was 0.78 µg/mL.

BRINE SHRIMP LETHALITY ASSAY (BSLA)

The leaf and stem extracts of *Cissus arguta* and *Cissus quadrangularis* were toxic to brine shrimp (Fig. 1). However, the crude methanol extract of *Cissus quadrangularis* stem was more toxic (LC_{50} = 10.62 µg/mL) compared to the *Cissus arguta* stem extract (LC_{50} = 139.20 µg/mL) while the crude methanol extract of *Cissus arguta* leaf was more toxic (LC_{50} = 31.57 µg/mL) compared to the *Cissus quadrangularis* leaf extract (LC_{50} = 106.20 µg/mL). The LC_{50} value of the positive control (Doxorubicin) against brine shrimp was found to be 10.90 µg/mL.

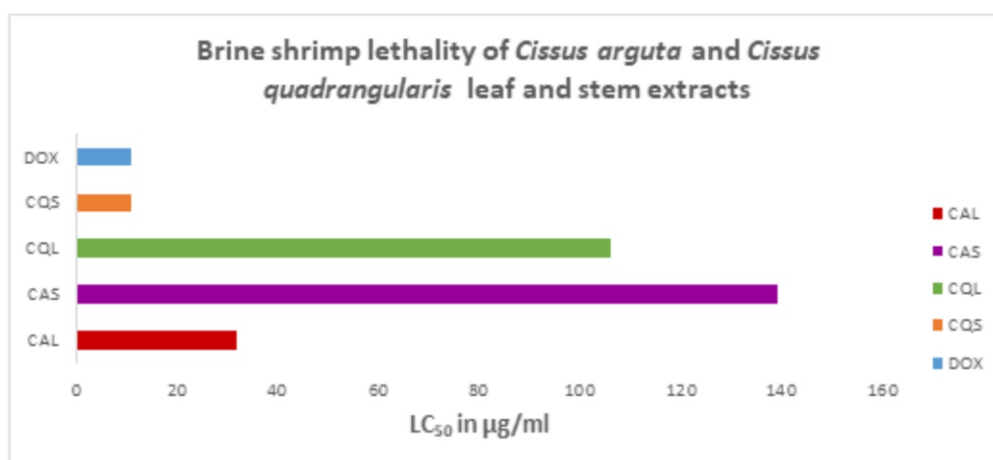


Fig 5: A chart comparing the brine shrimp lethality of crude methanol extracts of *Cissus arguta* and *Cissus quadrangularis* leaves and stems.

KEY: CAL = *Cissus arguta* leaf, CQL = *Cissus quadrangularis* leaf, CAS = *Cissus arguta* stem, CQS = *Cissus quadrangularis* stem, DOX = Doxorubicin

DISCUSSION

Cissus arguta and *Cissus quadrangularis* are ethno-botanically used in the treatment of various bacterial, fungal and viral infections [13, 19, 20]. These have been supported by the results of the present study which provide *in vitro* evidences for the antimicrobial activity of the two *Cissus* species. Other plants from this genus such as *Cissus ripens*, *Cissus populnea*, *Cissus cornifolia*, *Cissus vitiginea* and many others have also been reported to be effective against microbial infections [21, 22, 23, 24]. Stigmasterol and β -sitosterol which are antimicrobial compounds isolated from the stem extract of *Cissus populnea* have been found to exhibit good antimicrobial activity against methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *enterococcus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, *Trichophyton rubrum* and *Trichophyton mentagrophyte* [22]. Antimicrobial studies conducted by Maroyi [23] on *Cissus cornifolia* rootstock extracts using the broth microdilution method showed significant antibacterial and antifungal activities against the tested pathogens. Other species of the Vitaceae family, have also been seen as valuable sources of antimicrobial phytoconstituents [25, 26, 27]. The ethno-pharmacological knowledge and the chemotaxonomic relationship of *Cissus arguta* and *Cissus quadrangularis* to other species within the same genus and family suggest that their plant parts might be a good source of antimicrobial bioactive compounds.

The most commonly utilized method of antimicrobial susceptibility testing is the agar disc diffusion method. Here in this study, the broth microdilution method was employed to determine the minimum inhibitory and bactericidal concentrations of extracts. Studies have shown that the broth microdilution seems to be more appropriate when investigating the activity of botanicals [28]. It was also said that this method has several advantages compared to the agar diffusion method. The broth microdilution method is quantitative, less cumbersome, gives room for the use of small amounts of compounds or plant extracts as well as culture media, it is very convenient for the rapid screening of many samples and also yields reproducible results, and well adapted for systemic drug therapy [28, 29, 30].

The results obtained from this research showed a comparative analysis of the antimicrobial efficacy and brine shrimp lethality of the leaf and stem extracts of two species of *Cissus*. The minimum inhibitory and bactericidal concentrations of the methanol extracts of *Cissus arguta* and *Cissus quadrangularis* indicated their varying degrees of effectiveness against gram-positive and gram-negative bacteria with the stem extract of *Cissus quadrangularis* showing more promising antibacterial potentials than other extracts. It was noted by Aliannanis *et al.*, [31] that according to the minimum inhibitory concentration values recorded, the efficiency of antimicrobial activities of crude extracts could be evaluated. Extracts with MIC values lower than 50 $\mu\text{g/mL}$ are considered to be strong inhibitors, while those with values greater than 50 $\mu\text{g/mL}$ are moderate-weak inhibitors. The same can be said to apply to the minimum bactericidal concentration values. *Cissus arguta* and *Cissus quadrangularis* extracts showed significant activity against the gram-positive bacteria tested (*Staphylococcus aureus* and *Bacillus subtilis*), with MIC values ranging from 25 to 200 $\mu\text{g/mL}$ and MBC values from 0.78 to 200 $\mu\text{g/mL}$, as well as the gram-negative bacteria tested (*Klebsiella pneumoniae* and *Escherichia coli*) with MIC values ranging from 12.5 to 100 $\mu\text{g/mL}$ and MBC values from 25 to 100 $\mu\text{g/mL}$. This suggests

that the extracts can inhibit and kill gram-positive bacteria and gram-negative bacteria effectively.

Gram-positive bacteria have a thick peptidoglycan layer in their cell walls, which is the primary target for many antibiotics. The effectiveness of the extracts against gram-positive bacteria suggests that they may interfere with cell wall synthesis or disrupt the cell membrane [32]. Antibiotics like penicillin target the peptidoglycan layer, causing cell lysis. The extracts might have a similar mechanism, disrupting the integrity of the cell wall. The work of Kumar and Engle [32] underscores the potential of natural products to serve as effective agents against *Staphylococcus aureus* by disrupting the bacterial membrane and cell wall, which are critical for bacterial survival and pathogenicity. Studies have shown that *Cissus quadrangularis* has significant antibacterial activity against *Staphylococcus aureus*, with similar MIC values [33]. This supports the findings of the current experiment.

Gram-negative bacteria have a thinner peptidoglycan layer but possess an outer membrane that can act as a barrier to many antibiotics. The activity of the extracts against gram-negative bacteria suggests they might be able to penetrate this outer membrane or inhibit essential cellular processes. Antibiotics like ciprofloxacin inhibit DNA gyrase, an enzyme critical for DNA replication. The extracts might inhibit similar targets or disrupt the outer membrane. Research on other plants like *Boerhavia diffusa* has shown lower antibacterial activity against gram-negative bacteria compared to *Cissus quadrangularis* [34]. Studies carried out by Gnanarani *et al.*, [24] on *Cissus vitiginea* had earlier reported the significant antibacterial activity of *Cissus vitiginea* against gram-negative bacteria like *Escherichia coli* and *Klebsiella pneumoniae* with MIC and MBC values ranging from 50 - 200 $\mu\text{g/mL}$ and 100 - 400 $\mu\text{g/mL}$ respectively. Another study on *Cissus quadrangularis* activity against antibiotic-resistant bacteria reported MIC values for methanol extracts ranging from 0.19 to 3.13 mg/ml [19]. The latter is somewhat higher than the values observed in this study, suggesting more significant antimicrobial activities. Maximum activity was also observed in the ethanol, methanol and ethyl acetate extracts of *Cissus quadrangularis* stem against the gram-negative bacteria, *Klebsiella pneumoniae* [35]. A lower Minimum Inhibitory Concentration (MIC) value of a particular extract indicates that a smaller amount of the extract is required to inhibit the growth of a microorganism [36]. This generally means that the extract has a higher potency or effectiveness against gram-negative bacteria, and this might be attributed to active phytochemicals present in them.

The result of the comparative antimicrobial activity studies between the two *Cissus* species indicated that *Cissus arguta* leaf extract was more active than *Cissus quadrangularis* leaf extract while *Cissus arguta* stem extract was less active than *Cissus quadrangularis* stem extract. This result is also in relation with their toxicity to brine shrimp. All the plant extracts showed potential cytotoxic activity with varying levels of toxicity observed among the tested plant extracts. The LC_{50} values, which represent the concentration required to kill 50% of the brine shrimp population, range from 10.62-139.20 $\mu\text{g/mL}$ in comparison with doxorubicin having LC_{50} value of 10.90 $\mu\text{g/mL}$. A lower LC_{50} values indicate higher toxicity, and plants found to be toxic to brine shrimp are likely to be good candidates for anti-cancer research [37].

The stem extract of *Cissus quadrangularis* showed the highest cytotoxic activity with LC_{50} value of 10.62 $\mu\text{g/mL}$ while the stem extract of *Cissus arguta* showed the lowest cytotoxic

activity with LC₅₀ value of 139.2 µg/mL. The leaf extracts of *Cissus quadrangularis* and *Cissus arguta* exhibit moderate toxicity. The brine shrimp toxicity of these plant extracts may also suggest the synergistic activity of several bioactive compounds present in the extracts. These findings align with existing literature, which highlights the potent bioactive compounds in these plants, contributing to their medicinal properties and varying levels of toxicity [38]. For instance, *Cissus quadrangularis* is known for its anti-inflammatory and antioxidant activities [39], which may explain its high toxicity in the bioassay. While high toxicity in plant extracts may correlate with strong antimicrobial effects, it also raises significant concerns regarding safety and the therapeutic window [30]. Extracts with very low LC₅₀ values, such as *Cissus quadrangularis* stem at 10.62 µg/mL, might be effective at low concentrations but could pose a risk of toxicity to human cells. Various research always recommends careful dose optimization for such scenarios which is crucial to identify concentrations that are effective against microorganisms (low MIC and MBC) while remaining safe for human use. Also, calculating the selectivity index (SI), which is the ratio of cytotoxicity (LC₅₀) to antimicrobial activity (MIC), is essential. A high SI indicates that the extract is selectively toxic to microorganisms rather than human cells, which is desirable for therapeutic applications. Toxicological studies carried out on *Cissus quadrangularis* reveals that there is no toxic effect in its extract [40, 41]. There is also need for further studies on the toxicity index of *Cissus arguta*.

CONCLUSION

The methanol extracts of *Cissus arguta* and *Cissus quadrangularis* were shown to have varying levels of antibacterial and antifungal activity as well as cytotoxicity on brine shrimps. The extracts were more effective against the gram-positive and gram-negative bacteria than the fungal pathogens with *Cissus quadrangularis* stem extract exhibiting more significant antimicrobial and cytotoxic activity than the other extracts. The mechanisms may involve the disruption of cell walls or membranes and inhibition of essential cellular processes. However, further studies should determine the selective index of these extracts to know the exact dose that will inhibit bacterial activities without disrupting the body's cellular activities. These findings are consistent with existing literature, underscoring the potential of these plants as sources of new antimicrobial and cytotoxic agents.

MATERIALS AND METHODS

Plant Collection

The leaves and stems of *Cissus arguta* were collected from Amina Way, University of Ibadan, Oyo State, Nigeria, while the leaves and stems of *Cissus quadrangularis* were collected from Ajibode, Ibadan, Oyo State, Nigeria. *Cissus arguta* was identified and authenticated by Mr. T. K. Odewo at the Forest Herbarium Ibadan (FHI), a unit of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. A voucher specimen of the authenticated *Cissus arguta* plant sample was deposited at the Department of Pharmacognosy Herbarium, University of Ibadan (DPHUI), Oyo State, with a voucher number of FHI 113879. *Cissus quadrangularis* was identified by Mr. Olubunmi Mada, the Herbarium Curator of the Department of Pharmacognosy Herbarium, University of Ibadan, Ibadan, Nigeria.

Microbial Strains

The bacterial and fungal strains used for the study were pure cultures obtained from the Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Oyo State, Nigeria. The microorganisms were selected due to their medical relevance. The Microorganisms used for this study include two Gram-positive bacteria, *Staphylococcus aureus* (ATCC 29213), *Bacillus subtilis* (ATCC 6633), two Gram-negative bacteria, *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603) and two fungi (*Candida albicans*, *Trichophyton rubrum*).

Extraction of Plants

Plant samples were air-dried at a room temperature of 25 °C for two weeks and were pulverised. Weighed portions (200g) of the powdered leaves and stems plant samples were macerated with 100% distilled methanol for 72 hours with regular stirring. Extracts were filtered using a Buchner funnel with filter paper and concentrated *in vacuo*, using a Rotary evaporator [Rotavapor® R II, Buchi, Switzerland]. The filtrate was evaporated to dryness at room temperature (25 °C) to obtain crude methanol extracts.

Preliminary Phytochemical Screening

Phytochemical screening was performed on the powdered samples of *Cissus arguta* (leaf and stem) and *Cissus quadrangularis* (leaf and stem) using standard methods [42, 43] to screen for the presence of flavonoids, saponins, tannins, alkaloids, and anthraquinones.

Assay for antimicrobial activity

Preparation of extract stock solution

Methanol extracts (10 mg) from the plant samples were dissolved in 5 mL of methanol to make 2 mg/mL of stock solution and stored at 4 °C until use.

Preparation of Microbial Culture

Bacterial strains were sub-cultured into fresh nutrient agar (Oxoid (CMO405), England) plates and stored in a refrigerator at 40 °C for use. Well-isolated colonies of the same morphological type of each organism were enumerated in 5mL Tryptic Soy Broth and incubated at 37 °C for 16h before antibacterial testing to attain the turbidity of 0.5 McFarland standard (approximately 1.0 ×10⁶ CFU mL⁻¹ for *E. coli*). Similarly, the fungi were cultured overnight at 37 °C in Sabouraud Dextrose Agar.

Standard Antimicrobial Drugs

The standard drugs, 0.1% ciprofloxacin and 0.1% ketoconazole, were used as a positive control for antibacterial and antifungal activities respectively.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration was determined by the broth microdilution method using a sterile 96-well microtiter plate [2]. Stock solutions (2 mg/mL) of *Cissus arguta* and *Cissus quadrangularis* leaf and stem extracts were diluted with culture Tryptic Soy Broth (TSB) to a working concentration of 200 µg/mL. Serial two-fold dilutions of the working concentrations were made in the microtiter plate using TSB (in triplicate), to obtain concentrations ranging from 0.78 to 200 µg/mL. All controls including sterility control (broth and plant extract), control (containing culture broth and methanol,

without antimicrobial substance), positive control (ciprofloxacin and ketoconazole for bacteria and fungi respectively), and the negative control (broth and organism) were as well distributed in the 96-well plates. Each test and control well was inoculated with 20 µL (0.5 McF) of each bacterial and fungal suspension. All experiments were performed in triplicates and the micro-dilution plates were incubated at 37 °C for 24 h (bacteria) and 25 °C for 48 h (fungi). After incubation, 20 µL of 0.2 mg/mL p-INT alcoholic solution (p-iodonitrotetrazolium violet, Sigma®) was added to each well in the plates. The plates were further incubated at 37 °C for 30 min. Wells with a color change from yellow to pinkish red indicated microbial growth while clear/yellow coloration indicated no microbial growth. The MIC values were defined as the lowest concentration of each extract, which completely inhibited microbial growth.

Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) was determined using the microtiter plate used in the MIC determination. Tryptic Soy Broth in the wells of the microtiter plate which showed no microbial growth after incubation during the MIC assays was streaked separately on another Tryptic Soy Broth and incubated in an inverted position at 37 °C for 48 h. The MBC was regarded as the lowest concentration of the extract which did not produce any growth on the Tryptic Soy Broth after 24–48 hours of incubation.

Brine shrimp (*Artemia salina*) lethality assay (BSLA)

The Brine shrimp lethality assay was carried out according to the method of Ogbole *et al.*, [2]. Artificial seawater was prepared by dissolving sea salt in distilled water (40 g/L.) supplemented with 6 mg/L dried yeast. Brine shrimp eggs (*Artemia salina*) were hatched in artificial seawater during 48 hours of incubation in a warm room (22–29 °C). Brine shrimp larvae (nauplii) were collected with a Pasteur pipette. 20 mg of each of the extracts were dissolved in 2 mL of seawater. The stock solution for the methanol extracts (10 mg/mL) was made up to 1000 µg/mL as the starting concentration in the artificial sea and serially diluted into different concentrations (1000-1 µg/mL). Serial dilutions were made in vials in triplicate in 100 µL seawater. The negative control vials contained methanol in seawater without plant extract while Doxorubicin was used as the positive control. A suspension of nauplii containing 10 brine shrimp larvae (100 mL) was added to each vial using a Pasteur pipette. The number of survivors and deaths were recorded after 24 h.

Statistical Analysis

The 50% lethal concentration for each extract was calculated from concentration-effect curves after non-linear regression analysis using GraphPad Prism 8 software. The data obtained were expressed as the standard error of the mean of values obtained in triplicate from three independent experiments.

Author contributions: CRediT

TOA designed the work, EGM carried out the research, and drafted the manuscript; TOA supervised the study; TOA and OSA revised the manuscript. All authors read, made comments, and agreed on the final manuscript.

Declaration of Competing Interest

All authors declare that there is no conflict of interest related to

this article.

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List of non-standard abbreviations

- **CAL:** *Cissus arguta* leaf methanol extract
- **CAS:** *Cissus arguta* stem methanol extract
- **CQL:** *Cissus quadrangularis* leaf methanol extract
- **CQS:** *Cissus quadrangularis* stem methanol extract
- **FHI:** Forest Herbarium Ibadan
- **DPHUI:** Department of Pharmacognosy Herbarium University of Ibadan
- **BSLA:** Brine shrimp lethality assay
- **MIC:** Minimum inhibitory concentration
- **MBC:** Minimum bactericidal concentration
- **p-INT:** para-iodonitrotetrazolium

REFERENCES

1. Maraz, K. M., & Khan, R. A. (2021). An overview on impact and application of microorganisms on human health, medicine and environment. *GSC Biological and Pharmaceutical Sciences*, 16(1), 089-104.
2. Ogbole, O. O., Ndabai, N. C., Akinleye, T. E., & Attah, A. F. (2020). Evaluation of peptide-rich root extracts of *Calliandria portoricensis* (Jacq.) Benth (Mimosaceae) for *In vitro* antimicrobial activity and brine shrimp lethality. *BMC Complement Med Ther*, 20(1), 30.
3. Moyo, B., & Mukanganyama, S. (2015). Antibacterial effects of *Cissus welwitschii* and *Triumfetta welwitschii* extracts against *Escherichia coli* and *Bacillus cereus*. *International Journal of Bacteriology*, 2015(1), 162028.
4. Tenover, F. C. (2006). Mechanisms of antimicrobial resistance in bacteria. *American Journal of Infection Control*, 34(5, Supplement), S3-S10.
5. Céspedes, L. D., Fonseca, Y. M. C., Pérez, A. C., & Pérez, R. L. (2023). Current microbiological trends of microorganisms causing nosocomial infections. *Microbes and Infectious Diseases*, 4(4), 1150-1157.
6. Galappathie, S., Palombo, E. A., Yeo, T. C., Ley, D. L. S., Tu, C. L., Malherbe, F. M., & Mahon, P. J. (2014). Comparative antimicrobial activity of South East Asian plants used in Bornean folkloric medicine. *Journal of herbal medicine*, 4(2), 96-105.
7. Agboke, A., Nwosu, C., Obindo, D., Ekanem, M., Edet, E., & Ubak, I. (2020). Comparative Antimicrobial Activities of *Alchornea cordifolia* Leaf Crude Extracts and Cephalosporin Antibiotics on Some Pathogenic Clinical Isolates. *Journal of Drug Delivery and Therapeutics*, 10, 170-176.
8. Anibijuwon, I., Abioye, J., & Onifade, A. (2011).

- Comparative antimicrobial activities of some plant extracts and commercial antibiotics against some selected pathogens of food origin. *International Journal of Medicine and Medical Sciences*, 3(8), 268-272.
9. Mehta, R., Dhruv, S., Kaushik, V., Sen, K. K., Khan, N. S., Abhishek, A., Dixit, A. K., & Tripathi, V. N. (2020). A comparative study of antibacterial and antifungal activities of extracts from four indigenous plants. *Bioinformation*, 16(3), 267.
10. Méndez-López, L. F., Garza-González, E., Ríos, M. Y., Ramírez-Cisneros, M. Á., Alvarez, L., González-Maya, L., Sánchez-Carranza, J. N., & Camacho-Corona, M. d. R. (2020). Metabolic profile and evaluation of biological activities of extracts from the stems of *Cissus trifoliata*. *International Journal of Molecular Sciences*, 21(3), 930.
11. Fernandes, G., & Banu, J. (2012). Medicinal properties of plants from the genus *Cissus*: A review. *Journal of Medicinal plants research*, 6(16), 3080-3086.
12. Manokari, M., & Shekhawat, M. S. (2019). An updated review on *Cissus vitiginea* L. (Family: Vitaceae)-An important medicinal climber. *World News of Natural Sciences*, 22, 75-83.
13. Ogunmefun, S., Ajayi, T., & Moody, J. (2022). Anti-Inflammatory Activity of the Methanol Extracts of *Cissus populnea* Guills & Pers and *Cissus arguta* Hook. F. *Archives of Basic and Applied Medicine*, 10(2), 116-121.
14. Dickson, U. J., Igbe, I., & Edema, M. O. (2012). Antibacterial and antifungal activity and Bone healing Potency of methanol extract of *Cissus arguta* Hook F. *Nigerian Journal of Chemical Research*, 17, 1-11.
15. Sundaran, J., Vasanthi, M., Kamalopathy, M., Bupesh, G., & Sahoo, U. (2020). A short review on pharmacological activity of *Cissus quadrangularis*. *Bioinformation*, 16(8), 579.
16. Siddiqua, A., & Mittapally, S. (2017). A review on *Cissus quadrangularis*. *The Pharma Innovation Journal*, 6, 329-334.
17. Khan, N.A., & Baghel, U. S. (2023). A Review-Hadjod A Magical Remedy. *International Journal of Biology, Pharmacy and Allied Sciences*. 12(1), 484-498.
18. Mishra, G., Srivastava, S., & Nagori, B. (2010). Pharmacological and therapeutic activity of *Cissus quadrangularis*: an overview. *International journal of pharmtech research*, 2(2), 1298-1310.
19. Rattanasuk, S., Boongapim, R., Phiwthong, T., Phuangsricken, S., & Putthanachote, N. (2021). Antibacterial profile of *Cissus quadrangularis* extracts against antibiotic-resistant bacteria isolated from Roi et hospital. *International Journal of Pharmacology*, 17, 97-102.
20. Raj, S. J., & Joseph, B. (2011). Pharmacognostic and traditional properties of *Cissus quadrangularis* Linn-An overview. *Int. J. Pharma Bio Sci*, 2(1), 131-139.
21. Satish, N., Balan, A., Aswini, K., Kavyashree, R., Reddy, R., Swetha, S., Suryan, S., & Nagananda, G. S. (2022). In vitro Evaluation of Antimicrobial, Antioxidant and Cytotoxic Effect of Ethnobotanically Important Plant *Cissus repens*. *Journal of Medical Sciences*, 22, 119-127.
22. Alex-Asaolu, A., Uba, A., Birnin-Yauri, U. A., & Yusuf, A. J. (2024). Isolation and Characterization of Antimicrobial Constituent (s) from the Stem of *Cissus populnea* Guill. & Perr. *Drugs and Drug Candidates*, 3(1), 172-183.
23. Maroyi, A. (2025). A review of medicinal, chemical, and pharmacological properties of *Cissus cornifolia* (Baker) Planch. (Vitaceae family). *Journal of Applied Pharmaceutical Science*, 15(2), 001-010.
24. Gnanarani, V., Mahalingam, S., Kesavan, H., Neelan, Y. D., Lee, J., Chang, D. W., Bakthavatchalam, S., & Kim, J. (2024). Assessment of biological properties of a methanolic extract obtained from the stem of *Cissus vitiginea*. *Biomass Conversion and Biorefinery*, 14(4), 5677-5688.
25. Dube, N. P., Siwe-Noundou, X., Krause, R. W., Kemboi, D., Tembu, V. J., & Manicou, A.-L. (2021). Review of the traditional uses, Phytochemistry, and pharmacological activities of *Rhoicissus* Species (Vitaceae). *Molecules*, 26(8), 2306.
26. Opena, J. M., & Sotto, R. (2022). Environmental Conditions, Phytochemical Constituents, and Antibacterial Activities of Two Philippine Medicinal Vitaceae Species. *Journal of Tropical Life Science*, 12(1), 11-19.
27. Maiyama, S., Dahiru, H., Mohammed, M., Halilu, M., Ugwah Oguejiofor, C., & Babagana, A. (2023). Phytochemical Screening and In vitro Antimicrobial Activity of Methanolic Root Extract of *Ampelocissus grantii* (Baker) Blanch. (Vitaceae). *Bima Journal of Science and Technology* (2536-6041), 7(01), 25-32.
28. Tamokou, J. D. D., Mbaveng, A., & Kuate, V. (2017). Antimicrobial activities of African medicinal spices and vegetables. In *Medicinal spices and vegetables from Africa* (pp. 207-237). Elsevier.
29. Langfield, R. D., Scarano, F. J., Heitzman, M. E., Kondo, M., Hammond, G. B., & Neto, C. C. (2004). Use of a modified microplate bioassay method to investigate antibacterial activity in the Peruvian medicinal plant *Peperomia galioides*. *Journal of ethnopharmacology*, 94(2-3), 279-281.
30. Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C., & Bezirtzoglou, E. (2021). Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. *Microorganisms*, 9(10), 2041.
31. Aligiannis, N., Kalpoutzakis, E., Mitaku, S., & Chinou, I. B. (2001). Composition and antimicrobial activity of the essential oils of two *Origanum* species. *Journal of agricultural and food chemistry*, 49(9), 4168-4170.
32. Kumar, G., & Engle, K. (2023). Natural products acting against *Staphylococcus aureus* through membrane and cell wall disruption. *Natural Product Reports*, 40(10), 1608-1646.
33. Kashikar, N., & George, I. (2006). Antibacterial activity of *Cissus quadrangularis* Linn. *Indian journal of pharmaceutical sciences*, 68(2), 245-247.
34. Abhayarathna, A., & Mahendranathan, C. (2024). In-vitro antibacterial activity of *Boerhavia diffusa* leaf extract against *Staphylococcus aureus* and *Escherichia coli*. *Ceylon Journal of Science*, 53(1), 45-49.
35. Ruskin, R. S., Kumari, V. P., Gopukumar, S., &

- Praseetha, P. (2014). Evaluation of phytochemical, antibacterial and anti-cancerous activity of *Cissus quadrangularis* from South Western Ghats regions of India. *Int J Pharm Sci Rev Res*, 28(1), 12-15.
36. Rezaeian, S., Attaran, S., & Pourianfar, H. R. (2016). Time-kill Kinetics and Antibacterial Activity of Crude Methanolic Extract of *Thymus daenensis* celak. *Biomed Res*, 27(2), 489.
 37. Abu, T., Ogbole, O., & Ajaiyeoba, E. (2021). Radical Scavenging Activity, Brine Shrimp Lethality Assay and HPLC Analysis of Methanol Extracts of *Secamone afzelii*. *Nigerian Journal of Pharmaceutical Research*, 17(2), 233-244.
 38. Ramachandran, S., Vamsikrishna, M., Gowthami, K., Heera, B., & Dhanaraju, M. (2011). Assessment of cytotoxic activity of *Agave cantula* using brine shrimp (*Artemia salina*) lethality bioassay. *Asian J Sci Res*, 4(1), 90-94.
 39. Kumar, D., Guna, R., Abbirami, E., Gayathri, N., Karthik, R., & Sivasudha, T. (2019). Anti-oxidant and Anti-inflammatory Properties of Stem and Root Extracts of *Cissus quadrangularis* (Veldt Grape). *Asian Journal of Engineering and Applied Technology*, 8(1), 15-19.
 40. Rex M, C., & Ravi, L. (2020). A review on *Cissus quadrangularis* L. as herbal medicine. *Indian Journal of Natural Products and Resources (IJNPR) [Formerly Natural Product Radiance (NPR)]*, 11(3), 155-164.
 41. Kothari, S. C., Shivarudraiah, P., Venkataramaiah, S. B., Koppolu, K. P., Gavara, S., Jairam, R., Krishna, S., Chandrappa, R. K., & Soni, M. G. (2011). Safety assessment of *Cissus quadrangularis* extract (CQR-300): subchronic toxicity and mutagenicity studies. *Food and chemical toxicology*, 49(12), 3343-3357.
 42. Evans, W. C. (2009). *Trease and Evans' pharmacognosy*. 16th Ed, Elsevier Health Sciences, pp. 571-603,
 43. Gunavathy, S., & Sherine, H. B. (2019). Determination of heavy metals and phytochemical analysis of some selected medicinal plants. *Int. J. Sci. Res. in Biological Sciences*, 6(3), 87-96.