



Determination of Phytochemical and Antimicrobial Screening of the Stem of *Cissus quadrangularis* (Vitacea)

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Abstract Problem of multi-drug resistant strains of microbes sequel to the hazards associated with the use of synthetic anti microbial agents has increased investigations involving using plant extracts as possible alternative drugs. The phytochemicals determination of the methanol and n-hexane stem extracts of *C. quadrangularis* was carried out using standard methods. The presence of alkaloids (1.4%), tannins (10%), glycosides (11.2%), saponins (0.47%), and flavonoids (4.7%) were revealed. The antimicrobial activities of the methanol and n-hexane stem extracts of the plant were determined on the following microorganisms: *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus niger*, and *Escherichia coli*, using punched agar diffusion method. Diameter zone of inhibition were determined in triplicate after incubating at 37°C for 24 hours. It was revealed that both methanol and n-hexane extracts of *C. quadrangularis* are active to both gram-negative and gram-negative organisms with methanol having the greater activity than the n-hexane extract. The study therefore has contributed to the use of screened phytochemicals to cure diseases such as scurvy, menstrual disorder, otorrhoea, and epistaxis.

Keywords *Cissus quadrangularis*, Extracts, Phytochemicals, Elements, Antibacteria

Introduction

Over the years, many plant compounds have remarkable role in medicine. The incidence of many human diseases proving incurable or almost incurable has continued to draw attention of researchers in health discipline world over on plant compounds that have outstanding role in medicine. However, African continent is one of the continents endowed with the richest biodiversity in the world. Over 5000 different species of plant substances have been recognized to occur in these areas and many of them have been found to be useful in traditional medicine for prophylaxis and cure disease [1].

In Nigeria as well as other African countries, several roots, fruits, seeds, leaves, and backs of plants are used for different medicinal purposes. This continued investigation and phytochemical screening of plant products in search of additional therapeutic secondary metabolites had led to significant break-through in the field of pharmacology and pharmacognosy and has helped tremendously in the development of modern pharmacotherapeutic in Africa and other parts of the globe [2].

These procedures have shown that substances originally thought to be rare in occurrence are almost universally distributed in the plant kingdom [2]. Among the numerous plant species is *Cissus quadrangularis*. *C quadrangularis* is a succulent plant of the family *Vitacea* commonly found in the tropical area [3]. It was reported that the plant was



used for weight reduction, reducing blood glucose level, and serum lipid [4], shows gastro protective and hepatoprotective proportion as well as suppresses chronic ulcer [5] and increases fracture healing process [6].

The search for plant with vast array of medicinal properties is intensifying since they hold promise for the discovering of therapeutic agents. This present research was carried out to assess the phytochemicals in *C quadrangularis* and possible application of the methanol and n-hexane extracts for antibacterial and antifungal activities.

Materials and Methods

Plant Material

The fresh stem of *Cissus quadrangularis* was collected from a herbalist at Nnewi, Nnewi-South Local Government Area of Anambra State. The plant was identified by Mr Ozioko, A. a retired plant taxonomist of the department of Botany, University of Nigeria Nsukka.

Preparation of the Plant Material

The fresh stem was chopped into smaller pieces using a machete, air dried for two weeks in a shade to a constant weight. Thereafter was pulverized into powder using manual grinder. The powdered sample was stored in a screw capped plastic container for further use.

Oganoleptic Properties Determination

The colour, taste, texture, and odour of the pulverized sample were determined and recorded accordingly.

Extraction

Preparation of methanol and n-hexane extracts.

Conventional solvent extraction method was used.

100g of the pulverized test sample was submerged in a transparent plastic bottle containing 500ml of methanol and allowed to percolate for 48hours at room temperature, with intermittent vigorous agitation of the sealed bottle to enhance extraction. It was followed by filtration through white muslim piece to remove debris. The filtrate was then filtered with No1 Whatmann filter paper. The filtrate thereafter was pooled and dried in the water bath at temperature of 85°C. This procedure was repeated using n-hexane as extracting solvent.

Qualitative phytochemical Determination

The crude methanol and n-hexane extracts were evaluated for the presence of alkaloids, flavonoids, steroids, saponins, tannins, glycosides, acidic compounds, terpenoids, and carbohydrates using standard methods [7,8,9,10].

Quantitative phytochemical determination

Quantitative determination of the phytochemicals – alkaloids, tannins, cardiac glycosides, saponins, and flavonoids- were done using the methods outlined by Harborne [7], Obadoni and Ochuko [11], and El-Olemy et al [12].

Antimicrobial Screening

Preparation of the Media

Pure cultures of the test organisms- *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa*, *Basillus sbstilitis*, and *Aspergillus niger*- used in the study were obtained from the Microbiology laboratory of Chukwuemeka Odumegwu Ojukwu University, Anambra state. The microbes were grown in nutrient agar medium while the fungus was grown in sobourand agar medium. The media were prepared according to the method outlined by Wolfsang and Hilda [13].



Elemental Analysis

The levels of heavy metals- Pb, Cd and trace elements-K, Ca, Zn, Fe, Mg, Na, Cu and Cr- contents of *C quadrangularis* were determined by atomic absorption spectroscopy

Results

The results of the organoleptic, phytochemical, elemental and antimicrobial analysis of *C quadrangularis* stem are shown in tables 1-5 below.

Table 1: Organoleptic characteristic of the stem of *C quadrangularis*

Parameter	Inference
Colour	Pale green
Texture	Coarse powder
Taste	Astringent
Odour	Disagreeable

Table 2: Concentration of Phytochemicals in the stem of *C quadrangularis*

Phytochemical	Concentration (%)
Alkaloids	1.40
Tannins	10
Cardiac glycosides	11.20
Saponins	0.47
Flavonoids	4.70

Table 3: Antimicrobial Activities of the Crude methanol and n-hexane extracts of *C quadrangularis*

Organism	Zones of Inhibition (mm)		
	Methanol	n-hexane	Blank (water)
<i>Staphylococcus aureus</i>	14.37	8.39	NA
<i>E. coli</i>	13.19	7.32	NA
<i>Pseudomonas aeruginosa</i>	10.30	6.39	NA
<i>Basillus sbstilitis</i>	14.22	9.37	NA
<i>Aspergillus niger</i>	8.39	5.39	NA

NA = No activity.

Table 4: Heavy and trace element Composition of *C quadrangularis*

Element	WHO(1998), FAO(2001) $\mu\text{g/g}$	Concentration ($\mu\text{g/g}$)
K	10-100	67.50 \pm 0.16
Ca	3600-80000	39.50 \pm 0.64
Zn	-	12.61 \pm 0.29
Na	400-500	22.52 \pm 2.06
Fe	50-5000	7.50 \pm 1.39
Cu	100-300	0.50 \pm 0.08
Mg	100-300	30.60 \pm 0.18
Cr	<100	0.08 \pm
Pb		3.5 \pm 0.01
Cd		ND

ND =Not Determined.

Discussion

Plants with certain tastes and smells prevails in the treatment of distinct diseases, thus organoleptic properties of a plant can influence its differential medicinal use.



Table 2 above presented the results of the phytochemical analysis done on the plant extract. The phytochemical screening revealed the presence of alkaloids, tannins, cardiac glycosides, saponins, and flavonoids in variable concentrations. These phytochemicals are prerequisite of medicinal plants. Most of them has been associated with antimicrobial, anti-inflammatory, hepatoprotective, analgesic and bone healing properties.

The classes of phytochemicals detected in the plant sample as shown in table 2 have been reported to exhibit the therapeutic effects and have both antibacterial and antifungal properties and have been used to prepare some drugs [14]. Flavonoids have analgesic and anti-inflammatory activities. Flavonoids have been reported to diuretic and antifungal properties. Cardiac glycosides are naturally cardio active drug used in treatment of congestive heart failure and cardiac arrhythmia [15]. The antimicrobial activities of the crude methanol and n—hexane extracts of *C quadrangularis* is attributed to the presence of various phytochemicals in the plant.

The antimicrobial activities of the crude methanol extract and n-hexane extracts are shown in table 3. The crude methanol extract of the *C quadrangularis* showed better inhibitory action than n-hexane crude extract. The methanol extract has the highest activity on *Staphylococcus aureus* with inhibition zone diameter of 14.37mm and least activity on *Aspergillus niger* with inhibition zone diameter of 8.39mm. The result suggests that the crude methanol extract of *C quadrangularis* has high activity than n-hexane crude extract. The disparity in activities has to do with the different in the polarity of the solvents Methanol being a polar solvent extract more of the active principles than non-polar n-hexane.

The result of the elemental analysis shown in table 4 indicates that the concentration of all the essential elements- K, Ca, Zn, Na, Fe, Cu, Mg, and Cr in the plant are within the safety limits prescribed by the World Health Organization. Calcium is one of the major components of bones. It is required by human in growth and development of bones and for proper functioning of muscles and nerves [16]. The high calcium content has lend credence that the plant has bone healing and antiosteoporotic activities which are most cited use of the plant. It functions by increasing the early regeneration of bone tissue in fractured bones and increases bone density in osteoporotic condition. High magnesium content of *C quadrangularis* is an added advantage to its antiosteoporotic and bone healing activities. It has been reported that calcium supplemented with magnesium improves bone mineral density. Magnesium deficiency alters calcium metabolism and the hormones that regulate calcium resulting in osteoporosis and also magnesium prevents cardiovascular disease [17]. Potassium helps the kidney function normally. It is crucial to heart function and plays a key role in skeletal and smooth muscle contraction, making it important for normal digestive and muscular function too. Zinc is an essential trace element and plays an important role in various cell processes including normal growth, brain development, bone formation, and wound healing [18]. Iron is an essential element of haemoglobin in human body [19]. It facilitates the oxidation of carbohydrates, protein and fat to control body weight which is very important factor in diabetes. Chromium is an essential mineral that plays important role in how insulin helps the body to regulate blood sugar levels. It has reported that chromium supplement helps diabetic patients to lower blood sugar levels [20].

Conclusion

Cissus quadrangularis extract was found to contain some phytochemicals, which are bioactive components: alkaloids, tannins, cardiac glycosides, saponins, and flavonoids. The extract was found to show significant activity against common pathogens like *E coli*, *Bacillus subtilis*, *S. aureus*, *Pseudomonas aeruginosa*, and *Aspergillus niger*. The elements present in the plant which has their individual functions work in synergy with the bioactive components of the plant. These results showed the therapeutic activities of *C quadrangularis* against human pathogens. It has therefore given scientific prove on the claims of the medicinal activity of the plant.

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