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Identification of Phytochemical Constituents of the Methanolic extract of *Vitellaria paradoxa* Responsible for Antimicrobial Activity against Selected Pathogenic Organisms

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ABSTRACT

The antimicrobial activities and preliminary phytochemical screening of methanolic extract of *Vitellaria paradoxa* was performed against clinical isolates obtained from UITH which included *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger* and *Candida albicans*. Leaf extracts of *Vitellaria paradoxa* were prepared using methanol as solvent. The extracts were tested using agar diffusion and broth dilution method . *Escherichia coli* was resistant to all the extracts of *Vitellaria paradoxa* . Antimicrobial activity was recorded by the methanolic extract of *Vitellaria*

paradoxa with zone of inhibition of 19mm on *Candida albicans*. The minimum inhibitory concentration of the extract ranged from 40mglml to 160mglml for *Vitellaria paradoxa* leaf extract. Methanolic leaf extract of *Vitellaria paradoxa* are found to be bactericidal and fungicidal on *Staphylococcus aureus* and *Aspergillus niger*, *Candida albicans* respectively. *Vitellaria paradoxa* leaf extract contained saponin, tannins, flavonoid, phenolics, steroids, alkaloids, phlobatannins and glycoside.

The result from this study ,therefore suggest the possibility of using *Vitellaria paradoxa* extracts as antimicrobial agents, which can be a great asset to drug development for purpose of health care delivery in Nigeria

Key words: Antimicrobial activity ,Minimum Inhibtory Concentration, Phytochemical , *Vitellaria paradoxa.*

Introduction

Plants are known to be the source of many chemical compounds. Medicinal plants were used by people of ancient cultures without knowledge of their active ingredients. The common practice of taking crude extract orally is laden with hazards as the extracts may contain some toxic constituents. There is an ever increasing need to limit toxic clinical drugs [1].

In modern times, the active ingredients and curative actions of medicinal plants were first investigated through the use of European Scientific methods [2]. The most important ingredients present in plant communities turn out to be alkaloids, terpenoids, steriods, phenols glycosides and tannins [3].

The information obtained from extracts of medicinal plants makes pharmacological studies possible. The mode of action of plants producing therapeutic effects can also be better investigated if the active ingredients are characterized.

Infectious diseases are the leading cause of death worldwide. The clinical efficiency of many

existing antibiotics is being threatened by the emergence of multidrug resistant pathogens [4]. Bacterial pathogens have evolved numerous defense mechanisms against antimicrobial agents and resistance to old and newly produced drug is on the rise. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity [5,6] . There are several reports in the literature regarding the antimicrobial activity of crude extracts prepared from plants [7-11] .

Also, it was reported that the *Aloe vera* extracts was bacteriostatic while *Vernonia amygdalina* (Bitter leaf) have bactericidal effect on *Pseudomonas aeruginosa* and *Staphylococcus aureus* [12].

Plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines [13]. Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health care since ancient times. Over 50% of all modern clinical drugs are of natural product origin and natural products play a vital role in modern drug development in the pharmaceutical industry [14].

Vitellaria paradoxa (vernacular name: emi, igi ose(Yoruba); Kadanya (Hausa); osisi, ori, okwuma (Igbo); kochi (Nupe) belongs to the Sapotaceae family under the accepted new name *Vitellaria paradoxa* [15] common name: Shea butter. It has dark gray bark, rough, fissured and exudes white latex when cut. It is easily confused with *Lophra alata*. The leathery leaves are borne incluster at the ends of the twigs. They are rounded at both ends. The young leaves are densely hairy but the older ones are sparsely hairy, becoming smooth with age [15] . It is common decidous trees in the savanna areas of Africa. It is a major crop in Sudan,

Senegal, Nigeria and Chad.

Evidences on its wide use by the traditional clerics in treating some infections and diseases have prompted us to choose and confirm this plant for further evaluation in order to ascertain its antimicrobial potential to treat infections and diseases caused by some pathogenic microorganisms.

Materials and methods

Sources of materials

Fresh leaves sample of *Vitellaria paradoxa* were collected from the Compound of Nigeria Stored Product Research Institute, Ilorin. The plant samples were identified at the Herbarium unit of the Department of Plant biology, University of Ilorin, Ilorin, Nigeria. The microorganisms used were obtained from the clinical isolates from the Department of microbiology and parasitology laboratory of the university of Ilorin Teaching Hospital, Ilorin, Nigeria. The bacteria were maintained on Nutrient agar slant at 4^0c and fungi are maintained on potato dextrose agar slant at 4^0c. The isolates were subcultured unto fresh media at regular interval before use.

Preparation of plant extracts

The fresh plant materials were air-dried for a period of three weeks and four days and they were grounded into powder using mortar and pestle. The grinded leaves were sieved to get fine powder that was used for the extraction.

Cold ethanolic extract

Fifty grams of each of the powdered plant are weighed and introduced into different conical flask containing 250ml of ethanol. Each conical flask are then covered with aluminum foil and placed
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containing 250ml of ethanol. Each conical flask are then covered with aluminum foil and placed on mechanical shaker. The suspensions are shaked for 48hours at 190rev.per.min. Each extract were decanted and passed through different clean muslin cloth and later filtered with whatman fitter paper. The filterate obtained was evaporated to dryness at 50^0c and the residues obtained are kept in an aluminum foil.

Reconstitution and sterilization of extract

The dried residue was weighed into McCartney bottles and appropriate volume of distilled water was added to make a stock solution of 200mg/ml, for example 2000mg in 20mls of distilled water. The stock solution was then sterilized using 0.65 membrane filter by suction pump. The sterilized extract were stored inside McCartney bottle and kept in a refrigerator.

Standardization of inoculum

The standard method for preparing inoculum described by National Committee for Clinical Laboratory Standards(1990) was followed [16]. A sterile wire loop was used to pick five colonies of each of the test organism into different labeled test tubes containing 5ml nutrient broth. The broth culture was incubated overnight at 37^0C for the bacteria and room temperature for the unicellular fungi until a slightly visible turbidity compared to 0.5 Mcfarland standard (1.5 x 10^8CFU/ml).

Some spore of *Aspergillus niger* were picked with loop and drop in nutrient broth. It was incubated for 2 days at room temperature. Serial dilution was done and 10^{-3} was used as inoculum.

Antimicrobial assay of the plant extracts

Prepared sterile potato dextrose agar and Mueller Hinton agar plates were inoculated with standardized organisms of 0.1ml of a day old culture. Glass spreader was used in spreading the inocula evenly on the surface of the agar and excess are drained off. A sterile cork borer of 5mm diameters was used to make five (5) ditches on the plates. The bacteria were inoculated into

Mueller hinton agar while *Candida albicans* and *A. niger* were inoculated into potato dextrose agar.

Varying concentrations of the extracts 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml were prepared from the stock concentration of the extracts. 0.5ml of each concentration of the extracts were dispensed into each of the ditches in the plates that are appropriately labeled. The fifth ditch in the plates were picked as control by adding 0.5ml of appropriate solvent use for the different extraction. The plates were done in duplicates and left on the bench for few minutes for the extract to diffuse into the agar and later incubation at 37^0C for 24hours. After incubation the zone of clearance around each ditch was measured using a metric ruler by taking measurement of the zone of clearance around the ditch. The diameter of the cork borer was removed from the diameter of the zone of clearance and this made or represented the antibacterial activity measured or diameter of the zone of inhibition.

Phytochemical Screening of the Leaves Extracts

Phytochemical screening was done in order to detect the presence of plant constituents such as alkaloids, tannins, saponins, phenolics, phlobatannis, flavonoids and glycosides using the methods described by Odebiyi and Sofowora [17].

a. **Test for saponins**

Two milliliter of the aqeous and ethanolic extracts in a test tube was shaken for two minutes. Fronthing which persisted on shaking was taken as evidence for the presence of saponins.

b. **Test for Alkaloids**

Three milliliter of the ethanolic and aqueous extracts was stirred with 5ml of 1%HCl on a steam bath for twenty minutes. The solution obtained was cooled and filtered and the filtrate was added to few drops of Mayer's reagent/picric acid. A cream precipitate indicated the presence of alkaloid.

c. **Test for Phenolics**

Two drops of 5% ferric chloride were added to 5 ml of the ethanolic and aqueous extracts in a test tube. A greenish precipitate was taken as an indication of phenolics.

d. **Test for Tannins**

A volume of 1ml of freshly prepared 10% KOH was added to a volume of 1ml of the ethanolic extracts and aqueous extracts. The presence of a dirty white precipitate was taken as indication of tannins.

e. **Test for Steriods**

To a volume of 1ml of the extracts, five drops of concentrated $H_2SO_4$was added. Red colouration indicated the presence of steroids .

f. **Test for Phlobatanins**

To a volume of 1ml of the ethanolic and aqueous extracts, 1% HCl acid was added. A red precipitate was taken as the presence of phlobatannis .

g. **Test for flavonoids**:

To a volume of 3ml of the ethanolic and aqueous extract, 1ml of 10% sodium hydroxide was added. A yellow colouration indicated the presence of flavonoids.

h**. Test for Glycosides:**

To a volume of 3ml of the ethanolic and aqueous extract, 2ml of chloroform was added. H_2SO_4acid was careful added to form a lower layer .A reddish brown colour at interface indicated the presence of a steroidal ring.

Results

The screening for antimicrobial activity of the leaves of the plant used in this study revealed that the plant extracts had varying effects on the growth of the clinical isolates. All the plant extracts have inhibiting strength on the test organisms. The methanolic extract of *Vitellaria paradoxa* was found to be most effective with the highest zone of inhibition (19mm) .*Candida albicans* was found to be the most susceptible organism to all the extract while *Escherichia coli* was resistance to all the extract . The antimicrobial activity of the leaf extract of *Vitellaria paradoxa* on the test organisms are shown in Table 1.

Table 1 show that methanolic extract of *Vitellaria paradoxa* leaf had antimicrobial effect on the tested isolates. *Staphylococus aureus* was inhibited by all the varying concentration of the

extract. *Escherichia coli* was resistant to the extract which was indicated by no zone of inhibition. 200mg/ml concentration was the only concentration that was able to stop the growth of *Aspergillus niger*. *Candida albicans* was succeptible to the extract with the zone of inhibition that range from 5mm – 19mm.

TABLE 1: Antimicrobial effect of methanolic extract of *Vitellaria paradoxa* leaf on test organisms.

CONCENTRATION	**ORGANISM/ZONE OF INHIBITION (mm)**			
(mg/ml)	***Staphylococcus Aureus***	***Escherichia Coli***	***Aspergillus niger***	***Candida albicans***
200	16	NI	5	19
100	13	NI	NI	12
50	9	NI	NI	9
25	6	NI	NI	5
Control	NI	NI	NI	NI

NI = NO INHIBITION

CONTROL: Solvent only, without extract.

The MIC and MBC/MFC values obtained for the extract on *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger* and *Candida albicans* varied . For instance, the MIC values of 50mg/ml and 40mg/ml were obtained for methanolic extract of *Vitellaria paradoxa Staphylococcus aureus* and *Candida albicans.* These concentrations gave bactericidal and fungicidal effect after 24hours of incubation. They therefore regarded as the lowest concentrations of the extract sufficient to kill defined proportion of viable organisms at a specified period.

TABLE 2: Minimum inhibitory concentration and minimum fungicidal/bactericidal concentration of ethanolic leaf extracts on the tested organisms.

Test organisms	***Vitellaria paradoxa***	
	MIC	**MBC/MFC (Mg/ml)**
Staphylococus aureus	50	80
Aspergillus niger	120	160
Candida albicans	40	60

TABLE 3: Phytochemical analysis of methanolic of *Vitellaria paradoxa* leaf extract.

		Vitellaria paradoxa
S/N	**Active Component**	**Methanol**
1	Saponin	-
2.	Tannins	+
3.	Flavonoid	+
4.	Terpenoid	-
5.	Phenolics	+
6.	Steroids	+
7.	Alkaloids	+
8.	Phlobatannins	+

KEY + = Present
\- = Absent

Discussion

The result of this study have shown that the methanolic *Vitellaria paradoxa* possess antimicrobial effect on *Staphylococcus aureus.*

This antimicrobial activity exhibitor could be due to complimentary nature of the active principle Candida *albicans*, and *Aspergillus niger*. This may probably due to the insolubility or partial solubility of the active ingredients of the plants or it may be due to the presence of low concentration of diffusible water soluble active constituents. Oil are generally soluble in methanol and ethanol, it will make all soluble active components to dissolve in the solvent (methanol and ethanol) [18]. The solubility of some of the active ingredients in methanol has ethanol and enhances their inhibitory nature on the test isolates. The result shown that the higher the concentration of the plant extract, the higher the zone of inhibition and the lower the concentration of the plant extract the lower the zone of inhibition.

Candida albicans was the most susceptible organism among all the test isolate with zone of inhibition of 19mm to methanolic leaf extract of *Vitellaria paradoxa* (Table 1), This may suggest that the active ingredient in all the extracts penetrated the fungi cells to appreciable

degree and caused reduction in fungal growth. *Aspergillus niger* that is a fungi like *Candida albicans* is not succeptible as *Candida albians* because it might have ability to utilize the components of the extract.

The antimicrobial effect of medicinal plants on microorganisms may depend on the type of medium used to culture to microorganisms [19]. The antimicrobial agent may be incapable of diffusing through the cell wall or membrane of the microorganism as a result of the complexity in the organism's cell structure. Also, the organism may be resistant to the extracts which may be due to the possession of inclusion bodies or extracellular substances for example *Staphylococcus aureus* produces slime that inhibits phagocytosis and antimicrobial agent like vancomycin and teixoplain. It was recorded and shown that *Escherichia coli* was the most resistant organisms among the tested microorganisms because there was no zone of inhibition observed on the plate tested with methanolic, leaf extract of *Vitellaria paradoxa.*

Generally, antimicrobial activity of plants is affected by the nature of biologically active component present in the plant, the method of extraction of the plant as well as the extractant used. Excessive heating which often affect biologically active substances such as flavonoids essential oils and other heterogenous phytoconstituent present in the extracts may also influence their activity [20,21]

Antifungal agents with low activity against an organism have high MIC while antifungal agents with high activity give low MIC. . The MIC of the leaf of methanolic extract of *Vitellaria paradoxa* was relatively lower on *Staphylococcus aureus* and *Candida albicans*. The MIC of *Villetaria paradoxa* methanolic extract was high on *Aspergillus niger*. This means that antimicrobial substances in the extracts were bactericidal and fungicidal at higher concentrations of the extracts. The result of the MBC and MFC of this research work is in agreement with the observation reported by Olorundare *et al*.[22]

Study of the phytochemical screening of aqueous, methanolic and ethanolic extracts of the leaf of *Bambusa vulgaris* and *Vitellaria paradoxa* used in this study, showed that the extract contain secondary metabolites (Table 11). The presence of these biologically active substances may have been responsible for the anti-bacterial and antifungal activities reported in this particular work. The results of the study have shown that leaf of *Vitellaria paradoxa* possess

pharmacologically active component capable of inhibiting or stopping the growth of pathogenic microorganisms used. The result showed that the extracts from leaf of *Vitellaria paradoxa* can be better purified to manufacture drugs for use in the treatment of skin infection, stomach upset, candidaisis and other diseases causes by the tested isolates.

The methanolic leaf extract of *Vitellaria paradoxa* that shown the largest zone of inhibition on most of the tested isolates contain Tannins, flavonoids, phenolics, steroids, Alkaloids, phlobatannins and glycosides. Alkaloids have been reported to interrelate with DNA of microorganisms; Tannins inactivate microbial adhesins, enzymes, cell envelope transport protein[17]. Scalbert reviewed the antimicrobial properties tannins . He listed 33 studies which had documented the inhibitory activities of tannins up to that point. According to these studies, tannins can be toxic to filamentous fungi, yeast and bacteria [23] . This report might have been in support of the antimicrobial efficacy of methanolic leaf extract of *Vitellaria paradoxa.*

The primary benefits of using plant-derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are of then associated with synthetic antimicrobials.[15]

This finding is significant because most bacteria and fungi have been reported to be resistant to the action of most antimicrobial agent available. Therefore, the active components identified in the extracts should be purified. Secondary screening should be carryout on the purified active components and in vivo test should be carry out. The chemical structure of the active component of the plant extract should be determined for possible industrial synthesis.

In conclusion, the microbial activity of the extract of *Vitellaria paradoxa* could be enhanced if the component are purified caused. Research laboratories are therefore enjoined to work hand in hand with traditional herbal practitioners so that while the traditional healers from their historic knowledge provide preliminary information on the uses of medicinal plant, the scientific basis for the efficacy of the extracts and so that, proper advice can be given on how the drugs should be prepared and administered. This plant therefore holds a promise as a potential source of new drug for treating the diseases of which the test organisms are aetiological agents.

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