



**PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF N- HEXANE FRACTION OF
THE CRUDE METHANOL EXTRACT OF THE LEAVES OF CISSUS POLYANTHA
(VITACEAE)**

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ABSTRACT

The n- Hexane fraction of the crude methanol extract of the leaves of *Cissus polyantha* (family: Vitaceae) used in traditional medicine for the treatment of conjunctivitis and inflammatory condition was evaluated for its phytochemical constituents and antimicrobial activity. The antimicrobial activity was studied using cup plate agar diffusion and broth dilution techniques and the organisms tested are: *Escherichia coli*, *Staphylococcus aureus*, *Methicillin Resistant Staphylococcus aureus*, *Corynebacterium ulcerans*, *Shigella dysenteriae*, *Klebsiella pneumoniae*, *Candida albicans*, *Candida krusei*, *Candida tropicalis* and *Pseudomonas aeruginosa*. Phytochemical screening revealed the presence of steroids, triterpenes and flavonoids. The results of the antimicrobial susceptibility test showed that the fraction had activity against all the organisms tested (zone of inhibition 15-19 mm) with the exception of *Corynebacterium ulcerans*, *Pseudomonas aeruginosa*, and *Candida tropicalis*. The activity was found to be comparable to that of Sparfloxacin 5 µ /disc [a standard antibiotic] and Griseofulvin100 unit /disc [a standard anti-fungal]. This study showed the potential of n-hexane fraction of the methanol extract of *Cissus polyantha* as a source of antimicrobial agents.

KEYWORDS: Antimicrobial, *Cissus polyantha*, Agar well diffusion method, Phytochemical

INTRODUCTION

Medicinal preparations derived from natural sources, especially from plants, have been in widespread use since time immemorial. Ancient texts of India and China contain exhaustive depictions of the use of a variety of plant-derived medications [1]. In fact, plants remain the main source of medicines for a large proportion of the world's population, particularly in the developing world, despite the advent of the pharmaceutical chemistry during the early twentieth century, which brought with it the ability to synthesize a lot of medicinal drug molecules and allowed the treatment of previously incurable and/or life-threatening diseases [2].

Not surprisingly, chemically synthesized drug gained popularity and became the basis of pharmaceutical industry. Over the years, however, synthetic drugs have been plagued by unwanted side-effects and toxicity among other problems. In addition, the search for new drugs against a variety of illnesses through chemical synthesis and other modern approaches has not been encouraging. These factors, as well as the emergence of new infectious diseases, the proliferation of disorders such as cancer, and growing multidrug resistance pathogenic microorganisms, have prompted renewed interest in the discovery of potential drug molecules from medicinal plants. Methicillin resistant *Staphylococcus aureus* (MRSA), for

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instance has generally become a major problem in the society. More people in the U.S now die from MRSA infection than from AIDS [3], [4]. MRSA was responsible for an estimated 94,000 life-threatening infections and 16,650 deaths in 2005 [4]. This compared to 16,000 people that died of AIDS in US. MRSA was found to be responsible for 37% of fatal cases of blood poisoning in UK in 1999 up from 4% in 1991 [5]. Half of the *Staphylococcus aureus* infections in the US are resistant to penicillin, Methicillin, tetracycline and erythromycin [5]. MRSA is thought to have caused 1,652 deaths in 2006 in UK up from 51 in 1993 [6].

Plants are rich in a wide variety of secondary metabolite such as tannins, alkaloids, saponins and flavonoids which have been found *in vitro* to have antimicrobial properties [7].

Cissus polyantha is one of such medicinal plants used by traditional healers for the treatment of microbial related illness in Africa. It is a semi-woody climber belonging to the family Vitaceae. The sap from macerated leaves is used for the treatment of conjunctivitis [8].

Previous biological studies revealed that methanol extract of the leaf of the plant has analgesic and anti-inflammatory activities [9], while the crude methanol extract of the tuber of the plant was found to have antimicrobial activity [10]. The present study was therefore carried out to investigate the antimicrobial activity of the n-Hexane soluble fraction so as to validate some of its ethno-medicinal uses with a view to establishing wither the observed biological activity lies on n-Hexane fraction so as to give room for the isolation and characterization of the isolated compounds.

MATERIALS AND METHOD

Collection and identification of plant material

A whole plant of *Cissus polyantha* was collected wild from Turunku Igabi Local Government Area of Kaduna State in June, 2010. The plant was identified by Mallam M. Musa of the Herbarium Unit, Department of Biological Science, Ahmadu Bello University, Zaria by comparing with the existing specimen with voucher Number 616.

Extraction and preparation of plant material

The leaves were air dried and pounded. About 150 g of the powdered plant material was extracted with methanol using cool maceration method for ten days. The extract was filtered using Whatman No. 1 filter paper. The solvent was removed at reduced pressure to obtained 10 g of the crude methanol extract. The extract was suspended in water and successively partitioned using n-hexane,

chloroform, ethylacetate and n-butanol to afford n-hexane, chloroform and ethyl acetate soluble fractions respectively. The n-hexane fraction was used for the study.

Phytochemical screening of the n-Hexane fraction

The method of Trease and Evans [7] were employed to test for the presence of tannins, glycosides, anthraquinones and alkaloids, while the methods of Silva *et al.*, [11] were used to test the presence of steroids, terpenoids, flavonoids and saponins.

Antimicrobial study

Test organisms

The organisms used for this study were *Escherichia coli*, *Staphylococcus aureus*, Methicillin Resistant *Staphylococcus aureus* (MRSA,) *Klebsiella pneumoniae* *Corynebacterium ulcerans*, *Shigella dysenteriae*, *Candida albicans*, *Candida krusei*, *Candida tropicalis* and *Pseudomonas aeruginosa*. These organisms are hospital isolates obtained from Ahmadu Bello University Teaching Hospital, Shika-Zaria, Kaduna State.

Susceptibility test

The antimicrobial activities of n-hexane fraction of crude methanol extract of the plant were determined using stock concentration of 500 mg/ml. The microorganisms were maintained on agar slant. The inocula were prepared by inoculating the test organisms in nutrient broth and incubating them for 24 h at 37°C for bacteria, while for Fungi Saboraud dextrose broth was used and was incubated for 48 h. After incubation, the broth cultures were diluted to 1:1000 for the Gram-positive bacteria and 1:5000 for the Gram-negative bacteria. One milliliter of the diluted cultures was inoculated into a sterile molten nutrient agar at 45°C and poured into sterile petri dish. Similarly 1ml of the diluted fungal suspension was poured into sterile saboraud dextrose agar plates and the excess sucked up with Pasteur pipette. These were swirled gently and allowed to solidify. Wells were bored on the solidified inoculated nutrient agar plates using cork-borer number 4. The wells were filled respectively with equal volume of 0.1 ml of 500 mg/ml of the fraction making 50 mg/ml.

Standard disc (5 µg/disc) of Sparfloxacin and (100 units/disc) Griseofulvin for bacteria and for fungi respectively were placed on the agar and saboraud dextrose agar plates and serve as positive control. 30 min was allowed for the extract to diffuse into the

agar after which the plates were incubated overnight at 37°C for 24 h and 25°C for 48 h for fungi and bacteria respectively. At the end of incubation period, diameter of inhibition zone was measured and recorded. The inhibition zones with diameter of 12 mm and above were consider as an indication of antimicrobial activity [12].

Minimum Inhibitory Concentration (MIC)

MIC was determined using broth dilution technique [13], [14]. 0.5 g of the n-hexane fraction of the Methanol extract of the leaves of *Cissus polyantha* was weighed and dissolved into 10mls of the sterilized nutrient broth to obtain concentration of 50 mg/ml of the fraction in the broth, five test tubes were arranged in row, dilution of the fraction in the broth was done to obtain concentration of 50, 25, 12.5, 6.25 and 3.125 mg/ml. Using a sterile syringe, 0.1 ml of the microorganism suspension in normal saline was transferred into each dilution in the test tubes and the test tubes were incubated at 37°C for 24h for bacteria and 25°C for 48 h for fungi after which turbidity was observed in each test tube, the test tube with lowest concentration of the fraction of the extract showing clear solution was taken as the MIC.

Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) was determined using the method described by Rotimi *et al.*, [15] by assaying the test tubes content resulting from MIC determination. A loopful of the content of each tube was inoculated by streaking on solidified agar plate and then incubated at 37°C for 24h and 25°C for 48 h for bacteria and for Fungi respectively after which the plate were observed for microbial growth. The lowest Concentration of the sub-culture with no growth was considered as minimum bactericidal Concentration (MBC) [12].

RESULTS AND DISCUSSION

The results of the phytochemical screening of the n-Hexane fraction of the methanol extract revealed the presence of steroid and flavonoids (Table 1). The fraction showed antimicrobial activity with zone of growth inhibition ranging from 15-19 mm (Table 2). The lowest MIC (12.5 mg/ml) and MBC (25mg/ml) were recorded against *Staph. aureus* and MRSA (Table 3 and 4).

Table1: Results of Phytochemical screening of n-hexane fraction of the leaves of *Cissus polyantha*

Test	Inference
Alkaloids	–
Flavonoids	+
Anthraquinone	–
Steroids	+
Tanininn	–
Saponins	–

+ = present, - = absent

Table 2: Antimicrobial potency of the n-hexane fraction of crude methanol extract of *Cissus polyantha* against test microorganisms (zone of inhibition in mm)

Test organism	Zone of Inhibition (mm) (Hexane fraction)	Zone of Inhibition (mm) (Sparfloxacin)
<i>Escherichia coli</i>	15	15
<i>Staphylococcus aureus</i>	17	21
<i>Pseudomonas aeruginosa</i>	00	16
<i>Corynebacterium ulcerans</i>	00	00
<i>Klebsiella pneumoniae</i>	17	20
MRSA	15	13
<i>Shigella dysenteriae</i>	19	20

Table 3: Antifungal potency of the n-hexane fraction of crude methanol extract of *Cissus polyantha* against test fungi (zone of inhibition in mm)

Test Fungi	Zone of Inhibition (mm) (Hexane fraction)	Zone of Inhibition (mm) (Griseofulvin)
<i>Candida albicans</i>	15	0
<i>Candida ibrusei</i>	15	13
<i>Candida tropicalis</i>	0	0

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) of the Fraction against the Microbes

Test organism	Concentration mg/ml	MIC	MBC
<i>Escherichia coli</i>	25.00		50.00
<i>Staphylococcus aureus</i>	12.50		25.00
<i>Klebsiella pneumoniae</i>	25.00		50.00
MRSA	12.50		25.00
<i>Shigella dysenteriae</i>	25.00		50.00

Table 5: Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the Fraction against the Microbes

Test fungi	Concentration mg/ml MIC	MFC
<i>Candida albicans</i>	25.00	50.00
<i>Candida krusei</i>	25.00	50.00

The results of the preliminary phytochemical screening of the n-hexane fraction revealed the presence of steroids and flavonoids. Some of these phytochemical constituents have been shown to have antimicrobial property [16]. Flavonoids for instance are known to exert inhibitory effect on microorganisms [17; 18]. The plant extracts contained flavonoids this was confirmed by the work of Sani *et al.*, [9] as such it might serve as a potential source of analgesic and/or anti-inflammatory agent. While some flavonoids; have anti-tumour, antibacterial or antifungal property, some are used in domestic veterinary medicine, particularly in the form of ointment for its anti-hepatotoxic properties [20].

Staphylococcus aureus is known to play a significant role in skin diseases including superficial and deep follicular lesion [21], so the strong activity of the n-Hexane fraction indicates that the plant can be effective against skin infections causes by these organisms. The antimicrobial activity exhibited by this fraction is comparable to the one shown by the standard antimicrobial Sparfloxacin and the fraction seems to have a broader antifungal activity when compared to the Griseofulvin.

The activity of the n-hexane fraction of the methanol extract against *Candida albicans* and *Candida krusei* indicates that the plant can be used as a good source of anti- fungal agent, most especially for the treatment of those fungal diseases such as thrush, vaginitis and other conditions such as pulmonary and generalized infections including endocarditis caused by *Cadida albicans* [22].

CONCLUSIONS

Based on the finding of the present study, the n – hexane fraction of *Cissus polyantha* leaves contains chemical constituents with antimicrobial activities, which may serve as a lead for the synthesis of potent antimicrobial agent. The activity exhibited by the fraction against the tested organisms that are

associated with various infectious diseases, has further shown that the activity may be due to the phytochemical constituents presence in the n-Hexane fraction. Research is on-going to isolate, characterize and test compounds with antimicrobial activity from the n-hexane fraction. Therefore, the strong activity shown by n-hexane fraction against MRSA could pave away to getting potential drugs for the treatment of those deadly diseases causes by MRSA.

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