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Research Article

PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF THE CRUDE METHANOLIC EXTRACT OF THE LEAF OF *CISSUS CORNIFOLIA* BAKER (PLANCH) (FAMILY: VITACEAE)

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ABSTRACT

The antimicrobial activity of the crude methanolic extracts of the leaf of Cissus cornifolia were investigated using Cup-plate and agar Diffusion methods. The extract was tested against clinical isolates of Staphylococcus aureus, Streptococcus pneumoniae, Corynebacterium ulcerans, Pseudomonas fluorescence, Proteus vulgaris and Salmonella typhi. The extract showed activities against all the organisms tested (zone of inhibition: 20-26mm) with the exception of Corynebacterium ulcerans and Pseudomonas fluorescence. Phytochemical screening of methanol extract revealed the presence of flavonoids, steroids/triterpenes, tannins and saponins. This study showed that the leaves of Cissus cornifolia contains bioactive compounds with antimicrobial activity.

Key words: *Phytochemical, antimicrobial, Cissus cornifolia*

INTRODUCTION

Infectious diseases are among the major causes of death in developing countries. This has been attributed to poverty and multiple drug resistance. Bacterial resistance to almost all antimicrobial agents has been reported[1]. This resistance may be due to indiscriminate use of antimicrobial drugs commonly used in the treatment of infectious diseases[2]. This makes the need for a new generation antimicrobial drugs an urgent one. There is a renewed focus on the use of medicinal plants today.

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These plants serve as sources of novel bioactive compounds and candidates or lead molecules that could be integrated into drug development programme [3]. The plant *Cissus cornifolia* (Baker), planch is a species of the genera Cissus and family vitaceae. It is an annual, sub – erect herb with height of about 1.3m from the parent woody root base. It sends up an erect or sub erect shoot each year after the fires, flowers are greenish yellow appearing in the rocky shrubs and bush savannah in Ghana and Northern Nigeria. It is locally called Riigarbiri (the role of the monkey) or Tsuwaawuum biri among the Hausa speaking people of Northern Nigeria. The plant is also referred to by number of names e.g. sinkantora dagari (Ghana), and Sama oro Manika (Ivory Coast)[4].

Cissus cornifolia has wide array of uses in African Traditional Medicine amongst which is its use by the Fulani of Northern Nigeria as

a remedy for gonorrhea. Tanganyika use the leaf –sap as sedative agent, the root-decoction is also used for malaria; septic tonsil and pharyngitis [5]. The aim of this study is to investigate the antimicrobial activity of the methanol extract of the leaf of *Cissus cornifolia* to ascertain the scientific proof for the use of the plant as an antimicrobial agent. Previous phytochemical analysis of *C. cornifolia* revealed the presence of alkaloids, flavonoids, saponins, steroids/terpenoids, stilbenoids and tannins[6].

MATERIALS AND METHODS

Plant Material

The sample of the plant material was collected from Kufena, Zaria in the month of September, 2009 and was authenticated at the Herbarium unit of Biological Sciences Department, Ahmadu Bello University Zaria Nigeria where a voucher specimen (No. 024) was previously deposited. The leaves were separated manually from the branches and air dried under shades. It was then made into powder using pestle and mortar.

Preparation of Extract

The powdered leaves (1600g) ware extracted using cold maceration with occasional shaking using 70% methanol for seven days. The extract was filtered and concentrated *in vacuo* to yield a residue referred to as the Crude methanol extract (CME) with a yield of 68.25g.The methanol extract was partitioned successively with, hexane, Chlorofom; and ethylacetate to give hexane (8.38g), Chloroform (4.5g), and ethylacetate (7.5g) fraction respectively.

Phytochemical Screening

CME was subjects to Phytochemical screening using standard procedure [7] to test the presence of alkaloids, flavonoids, tannins and saponins.

ANTIMICROBIAL SCREENING

The Micro organisms

The organisms used were obtained from the Medical Microbiology Department Ahmadu Bello University Teaching Hospital, Zaria Nigeria. All the bacterial cultures were checked for purity and maintained on a blood agar slant. The organisms are *Staphylococcus aureus, Streptococcus pneumoniae Corynebactereum ulcerans, Pseudomonas fluorescence, Proteus vulgairs and Salmonella typhi*.

Antimicrobial Testing

Preliminary antimicrobial activities of the extract were carried out using disc diffution and broth dilution methods. 0.2g of the extract was weighed and dissolved in 10mls of sterile distilled water to obtain a concentration of 20mg/ml. Blood agar was used as the growth medium and was prepared according to manufacturer's instruction, boiled to dissolve and was sterilized at 121^0C for 15mins, the medium was cooled to 45^0C and 20mls of the sterile medium was poured into sterilized petridishes seeded with 0.1ml of the standard inoculum of the test microorganisms. The inoculum was spread evenly by the use of a sterile swab over the surface of the medium. The seeded plates were allowed to dry in an incubator at 37^0C for 30mins. A standard cork borer of 6mm diameters was used to cut cups (well) at the centre of each inoculated medium and 0.1ml of the solution of the extract was introduced into each well on the medium, the plates were incubated at 37^0C for 24hrs after which the plates were observed for zones of inhibitions of growth. The zones were measured with a transparent ruler and the result recorded in millimeters. Controls were set up in parallel using the solvents that were used to reconstitute the extract. The plates were also observed for zones of inhibition after 24 h. The effects were compared with those of sparfloxacin at a concentration of 100 µg/disc.

Minimum Inhibitory Concentration (MIC)

The MIC was determined on the organisms that were sensitive to the extract and was done by Broth dilution method[8]. Nutrient broth was prepared according to manufacturer's instruction. 10mls 0f the medium was dispensed in a screw-capped tests tubes and were sterilized at 121^0C for 15mins. The broth

was then allowed to cool. MC. Farland turbidity standard scale No. 05 was prepared by adding 9.9mls of 1% $BaCl_2$ solution to give a turbid suspension of the microorganism. The broth cultures were diluted with the normal saline continuously until the turbidity matched that of the MC. Farland scale by visual comparism. At this point the microorganism had a concentration of about $1.5x10^8$ cfu/ml. Two-fold serial dilution of the extract with the broth was done to give concentrations of 20mg/ml. 10 mg/ml, 5 mg/ml, 2.5 mg/ml and 1.25 mg/ml. The initial concentration was obtained by dissolving 0.2g of the extract in the different concentrations of the broth. 0.1 ml of the standard inoculums of the microbes ware inoculated into the different concentrations of the extract in the test tubes. The tubes were then incubated at 37 ^{0}C for 24hrs, after which the test tubes were observed for turbidity (growth). The lowest concentration of the extract in the test tube which shows no turbidity was recorded as the Minimum Inhibitory Concentration.

Minimum Bactericidal concentration (MBC)

The tubes preceding MIC in the serial dilution were sub-cultured into the prepared medium by dipping a sterile wire loop into each test tube and streaking the surface of the labeled blood ager plates. The plates were then incubated at 37^{0}C for 24hrs after which they were observed for colony growth. The MBC was the plate with the lowest concentration of the extract without colony growth.

RESULTS AND DISCUSSION

PHYTOCHEMICAL SCREENING

The Phytochemical screening revealed the presence of saponins flavonoids, tannins and alkaloids (Table 1). Flavonoids and tannins have been reported to posses antimicrobial activity[9].

Table 1: Phytochemical screening of crude methanol extract of *Cissus Carnifolia*

Phytochemical Constitute	CME
Flavonoids	+
Steroids/terpenoids	+
Saponins	+
Alkaloids	+
Tannin	+

*Key + = Positive, CME = crude methanol extract

Table 3: ZONES OF INHIBITION OF EXTRACT AND STANDARD DRUG AGAINST TEST ORGANISMS

Test organism	**Mean zone of inhibition (mm)**	
	Methanolic extract	**Sparfloxacin**
S. aureus	**24**	**30**
S. pneumonia	**26**	**32**
C. ulcerans	**00**	**29**
P. flourescense	**00**	**20**
P. vulgaris	**20**	**22**
S. typhi	**22**	**25**
Drug Conc. = 0.2mg/ml.		

Table 2: MIC and MBC of crude methanol extract of *Cissus cornifolia* against the microbes (mgml^{-1})

Methenol Extract		
Test Organism	**MIC**	**MBC**
S.Aureus	5	10
S.pheumoniae	5	10
C.Ulcerans	-	-
P.Flourescense	-	-
P.Vulgaris	5	10
S.Typhe	5	10

*Key: - Not determined,

Discussion

The result of preliminary phytochemical screening of the crude methanol extract revealed the presence of flavonoids, tannins, steroids/triterpenes and saponins (Table 1). Flavonoids and tannins have been reported to possess antimicrobial activities[9]. Similarly, [10] reported that Flavonoids have anti-tumour, antibacterial and anti-fungal activities. The antimicrobial activity of flavonoids is due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall. While that of tannins may be attributed to their ability to inactivate microbial adhesion of enzymes and cell envelop proteins[9]. The saponins and flavonoids have been reported by several researchers [11,12,13,14,15] to be responsible for sedative and activity of the plant in mice; Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications [16]. Therefore, the antimicrobial activity of *Cisssus cornifolia* might be associated with the presence of these phytochemical constituents. Sparfloxacin, a standard antibiotic was found to be active on all the microorganisms tested with zone of inhibition ranging from 20 to 32. These values when compared with that of the CME showed that the activities of extract on the test organisms with the exception of C. ulcerans and P. fluorescence is very significant.

The CME showed strong inhibitory activity against all the organisms tested with the exception of *Pseudomonas fluorescence and Corynebacterium ulcerans* with zones of inhibition ranging from 20-26mm. The extract showed strong activity against *Streptococcus pneumoniae* with zone of inhibition of (26mm), *Staphylococcus aureus* (24mm), *Salmonella typhi* (22mm) and *Proteus vulgaris* (20mm). The crude methanolic extract was found to have MIC values of 5mg/mL for all the organisms and MBC values ranging from 10-20mg/mL. The MBC test showed scanty colonies growth on *P.vulgaris* and a heavy colonies growth on all the organisms except *S. pneumoniae and C. ulcerans* in which no colony growth was found.

The strong activity of the extract against *Staphylococcus aureus,* which was recognized as one of the major causes of infections in humans, occurring in both communities and hospitals[17], shows that the leaves extract of *Cissus cornifolia* can be a source of compounds that can be used to combat infections caused by this organism. For example, *Staphylococcus aureus* is known to play a significant role in skin diseases[18]. Hence the CME of the leaves of *Cissus*

cornifolia can be a source of compound(s) that may be effective in the treatment of skin infections. The extract also showed strong activity against *S. typhi*. The incidences of enteric fevers caused by this organism have reached a worrisome scale, especially in infrastructurally scarce environment due to the prevalence of drug resistance strains[19]. So this plant can be used to treat this worrisome condition.

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