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IN-VITRO ANTIMICROBIAL ACTIVITY OF *CHROZOPHORA BROCCHIANA* DIFFERENT EXTRACTS

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ABSTRACT

The methanol, ethyl acetate and hexane extracts of leaves, stem and oilseed of *Chrozophora borcchiana* were evaluated for antibacterial and antifungal activities. Streptomycin (STR) was used as standard for antibacterial assay. The leaves and stem of the plant were dried and extracted using three different solvents (methanol, ethyl acetate and hexane), while the oil was extracted with methanol only. The obtained extracts were tested for their antimicrobial activity against three Gram-positive bacteria (*Bacillus cereus*, *Bacillus subtitles*, *Staphylococcus aureus*, and three Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and Salmonella), and two fungals (*Candida albicans* and *Aspergillus flavus)* species using agar well diffusion method. The leaves and stem methanolic extracts were active against all the investigated bacterial and fungal strains, while leaves and stem ethyl acetate extracts were found to be less active as antimicrobial. The oil methanolic extract was active against *Bacillus cereus* and *Staphylococcus aureus* only.

Keywords: Antimicrobial activity, Methanol extract, Ethyl acetate extract, Hexane extract and *Chrozophora borcchiana*.

INTRODUCTION

Research work on medicinal plants is intensified and information on these plants be exchanged. Scientist's worldwide care about the scientific exploration of medicinal plants for the benefit of human being. A long times, plants have been a veritable source of drugs; man tends to ignore the importance of herbal medicine [1].

Polyphenols are naturally occurring secondary metabolites in all plant materials, and prominently found in herbs, vegetables, fruits, and seeds [2]. The most accruing polyphenols are flavonoids, which are benzo-γ-pyrone derivatives consisting of phenolic and pyrane rings, that can be divided into six classes including flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanidins [3,4]. The other common non-flavonoid polyphenols are phenolic acids. Owolabi et al [5] reported that, medicinal plants play a key role in health care with about 80% of the world populations relying on the use of traditional medicine which is predominantly based on plants. Mariod et al. [6] investigated wood extracts of *combretum hartmannianum, acacia seyal* and *terminalia brownie* for their antimicrobial screening. They reported that the ethyl acetate extract gave the highest zone of inhibition against Salmonella, and all other extracts showed moderate zones of inhibition against all the bacteria tested.

Chrozophora brocchiana (Vis.) Schweinf, family Eupharbiceae occurs from Cape Verde and Mauritania throughout the Sahel region east to Sudan, and is also found in Algeria and Egypt. In eastern states of Sudan the boiled seeds are used for food, and extraction of the oil is done by using traditional mills pulled by animals such as camels or cows [7].

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The analysis of the fatty acid composition of the *Chrozophora brocchiana* seed oil showed linoleic acid as the major component, followed by palmitic, oleic and stearic acids [8]. In the Hoggar region of Mali and Niger, the plant ash is applied to sores of humans and camels. In Niger the Hausa people rub crushed leaves on the affected sites to treat stitch in the side. The aerial parts are taken in decoction to strengthen lactating mothers and their children, and to treat fever and dysentery. In Benin powdered dried leaves in water are taken to treat diarrhoea. Root sap in water is used as ear drops to treat otitis [8]. In Senegal the plant is not browsed by most stock, except occasionally by sheep and goats, as it causes vomiting and diarrhoea. In Niger though, it is sought after by goats and at certain times of the year also by cattle. It is not suitable for making hay or silage. In central Sudan sweet, non-drying oil is pressed from the seeds.

To our knowledge no any serious study have looked at the antimicrobial properties of *Chrozophora brocchiana* extracts. This study is aimed at a preliminary evaluation of *Chrozophora brocchiana* different extracts for their potential antimicrobial activity

MATERIALS AND METHODS

Collection and preparing of plant materials

The fresh leaves, stems and seeds of Chrozophora plant were collected From Ghibaish, North Kordofan state, Sudan. The plant materials were air-dried in the laboratory and then ground into powder form using a mortar and sieving and then stored in airtight bottles. The oil was extracted by hexane from the ground seeds using Soxhelt.

Preparation of phenolic extracts

The phenolic compounds of leaves and stem were extracted following the method Parekh et al [10] with slight modification. In brief: 100 g of the dried ground leaves and stem of *C. brocchiana* was taken in 500 ml of methanol 80% (v/v), ethyl acetate and hexane, respectively and separately in conical flask, plugged with cotton wood and then kept on a rotary shaker at 220 ppm for 16 h, then the yield was collected using a filter paper. This procedure was repeated twice. The solvents were removed using a rotary evaporator and the final volume was taken (in table) then the final yield was stored at -8°C in a dark bottle, till analysis.

Phenolic Compounds-free Oil

Phenolic compounds-free oil was obtained by extracting phenolic compounds from the crude oil following the method of Tsimidou et al [11]. In brief, 50 g oil was dissolved in 50 mL petroleum ether using a 250-mL extraction funnel, and then was extracted three times with 30 mL of a mixture consisting of methanol:water (60:40, v/v). The lower layers containing phenolic compounds were removed. The petroleum ether was evaporated in a rotary evaporator at 40°C to obtain phenolic compounds-free oil. Samples of phenolic compounds-free oil were kept at -8°C for further analysis

Preparation of concentration of the extract

Gram of each crude extracts was dissolved in 10ml sterile distilled water to prepare four concentrations of the test extracts (0.1- 0.01- 0.001- 0.0001)

Test Strain and Culture Media

Strains of bacteria and fungal were obtained from ATCC (American Type Culture Collection). Antimicrobial activity of plant extracts against *B. subtilis'* DSM618, *B. cereus* MK 131, *S. aurous* ATCC 29213, *E. coli* (ATCC 25922), *P. aeruginosa* ATCC 27853, *S. typhi* laboratory isolates, *C. albians* (ATCC90028) and *A. flavus*- laboratory isolates-was studied.

The species of bacteria were grown in nutrient agar (HIMEDIA M001) and fungal species in Sabouraud dextrose (HIMEDIA M063). The concentration of bacteria suspensions was adjusted by using Macfrland SHD turbidity Tube. All bacterial strains were cultured aerobically at 37°C in nutrient broth and agar medium. Before the susceptibility test, cultures from solid mediums were sub-cultured in liquid media, incubated for 18 h and used as the source of inoculums for each experiment.

A standard antibiotic disc of Streptomycin was used as control at concentration of 106cfu/mL was used for inoculating nutrient agar plates for antibiotic sensitivity disc. All tests were carried out in quadruplicate.

Assay of Antibacterial Activity using Agar Well Diffusion Method

The antimicrobial activity of to the plant extracts to the test organisms was screened by using the agar well diffusion method Perez et al with a slight modification. The 25 ml of sterilized nutrient agar was poured into sterile petriplate, after solidification (28 g of nutrient agar in 1000ml sterile distilled water for bacterial and 65g of Sabouraud dextrose in 1000 ml sterile distilled water for fungi). 100µl of the fresh culture microorganism were swabbed on the respective plates. The well was prepared in the plates with the help of a cork-borer (1 cm) then 100µl of the test extracts introduced into the well and were allowed to stand on the bench for 1 h for proper diffusion. The plates were incubated overnight at 37°C for bacterial, and 48 h in 30°C for fungi (memmert). Microbial growth was determined by measuring the diameter of zone inhibition. The results were obtained by measuring the diameter in tables.

RESULTS

The antimicrobial activity of methanol, ethyl acetate and hexane extracts of leaves and stem, and oil methanol extract of *Chrozophora borcchiana* plant were observed using agar well diffusion method by measuring the diameter of the growth inhibition zone.

The results are showed in tables (1-3). The methanol extract of leaves showed a positive significant antibacterial and antifungal activity against all the test organism with inhibition zone between (1.5-2.5 cm) at 0.1-.0.01 concentration, and showed highest significant at all concentration against *S. aureus*. The ethyl acetate extract of leaves showed a positive significant antimicrobial activity against *B. cereus* with inhibition zone 1.5 cm at concentration 0.1mg and *S. areus* with inhibition zone between (3.0-2.3) at all concentration and resistant to fungal.

The hexane extract of leaves showed appositive significant antimicrobial activity against *B. subtitile* with inhibition zone (1.1-2.0 cm) at the concentration (0.1-0.01 mg) of extract and *S. areus* with inhibition zone between (1.5-2.5) in the same concentration and resistant to fungal.

The methanol extract of stem showed a positive significant antibacterial and antifungal activity against the entire test organism with inhibition zone between (1.5-2.5 cm) at 0.1mg concentration. The ethyl acetate extract of stem showed a positive significant against *S. areus* with inhibition zone 1.8 at 0.1 concentration and resistant to other organism. The hexane extract of stem showed a positive significant against *S. areus* with inhibition zone 1.5 at 0.1 concentration and resistant to other organism.

The methanol extract of oil showed a positive significant antimicrobial activity against *B. subtitile* with inhibition zone between (1.0 and 1.2 cm) at 0.1- .0.1 mg concentration, and *S. areus* with inhibition zone 1.2-1.5 at all concentrations and resistant to other organisms.

Table 1. The zone of inhibition diameter of *Chrozophora borcchiana* leaves extract*

Microorganism	Extract concentration	Zone of inhibition A	Zone of inhibition B	Zone of inhibition C	STR
Bacillus cereus	0.1 0.01 0.001 0.0001	2.1 1.6 R R	1.5 R R R	R R R R	3.0
Bacillus subtitles	0.1 0.01 0.001 0.0001	1.8 1.6 R R	R R R R	2.0 1.1 R R	NT
Staphylococcus aureus	0.1 0.01 0.001 0.0001	2.5 1.9 1.6 1.5	3.0 2.3 1.8 1.5	2.5 1.5 R R	1.8
Escherichia coli	0.1 0.01 0.001 0.0001	1.2 R R R	R R R R	R R R R	1.9
Pseudomonas aeruginosa	0.1 0.01 0.001 0.0001	1.8 1.3 R R	R R R R	R R R R	1.8
Salmonella	0.1 0.01 0.001 0.0001	1.6 1.2 R R	R R R R	R R R R	NT
Candida albicans	0.1 0.01 0.001 0.0001	1.5 R R R	R R R R	R R R R	NT
Aspergillus flavus	0.1 0.01 0.001 0.0001	1.5 R R R	R R R R	R R R R	NT

** A =Methanol extract, B=Ethyl acetate extract, C=Hexane extract, STR Streptomycin NT: Not Tested*

Table 2. The zone of inhibition diameter of *Chrozophora borcchiana* stem extract*

Microorganism	Extract concentration	Zone of inhibition A	Zone of inhibition B	Zone of inhibition C	STR zone
Bacillus czereus	0.1 0.01 0.001 0.0001	2. R R R	R R R R	R R R R	3.1
Bacillus subtitles	0.1 0.01 0.001 0.0001	2.3 R R R	R R R R	R R R R	NT
staphylococcus aureus	0.1 0.01 0.001 0.0001	2.5 R R R	1.8 R R R	1.5 R R R	1.8
Escherichia coli	0.1 0.01 0.001 0.0001	2.0 R R R	R R R R	R R R R	1.9
Pseudomonas aeruginosa	0.1 0.01 0.001 0.0001	2.5 R R R	R R R R	R R R R	1.8
Salmonella	0.1 0.01 0.001 0.0001	2.0 R R R	R R R R	R R R R	NT
Candida albicans	0.1 0.01 0.001 0.0001	1.5 R R R	R R R R	R R R R	NT
Aspergillus flavus	0.1 0.01 0.001 0.0001	2.0 R R R	R R R R	R R R R	NT

** A =Methanol extract, B=Ethyl acetate extract, C=Hexane extract, STR Streptomycin NT: Not Tested*

Table 3. The zone of inhibition diameter of *Chrozophora borcchiana* oil extract*

Microorganisms	Extract concentration	Zone of inhibition A	STR zone
Bacillus cereus	0.1 0.01 0.001 0.0001	1.2 1.0 R R	3.0
Bacillus subtitles	0.1 0.01 0.001 0.0001	R R R R	NT
Staphylococcus aureus	0.1 0.01 0.001 0.0001	1.5 1.5 1.2 1.2	1.8
Escherichia coli	0.1 0.01	R R	1.9

	0.001 0.0001	R R	
Pseudomonas aeruginosa	0.1 0.01 0.001 0.0001	R R R R	1.8
Salmonella	0.1 0.01 0.001 0.0001	R R R R	NT
Candida albicans	0.1 0.01 0.001 0.0001	R R R R	NT
Aspergillus flavus	0.1 0.01 0.001 0.0001	R R R R	NT

*** A =Methanol extract, STR Streptomycin NT: Not Tested*

DISCUSSION

The antimicrobial screenings are recorded in Tables 1-3 expressing the zones of inhibition of bacterial and fungal growths. It is interesting to note that the leaves and stem extracts are more effective against bacteria than fungal especially methanol extracts. While the oil extract was found to be effective against pathogenic bacteria.

Successful prediction of antibacterial activity from plant material is largely dependent on the type of solvent used in the extraction procedure. The methanol, follow by ethyl acetate and hexane extracts showed considerable amount of inhibition against *B. subtilis*, *B. cereus*, *E. coli*, *S. typhi*, and *P. euroginosa* respectively; with much activity on *S. aureus*. Many *S. seureus* strains are well known for their high antibiotic resistance against different antibacterial agents [12]. The activity also targeted non-pathogenic strains *B. subtilis*. This may be due to better solubility of the active components in organic solvent [13].

All the extracts had appreciable activities against *Aspergillus flavus* and *Candida albicans*. The standard antibiotics discs used in this study inhibited the growth of the test bacteria but inactive against fungal organisms. The zones of inhibition produced by STR discs against *Bacillus cereus* and *E. coli* were found to be higher than those produced by some extracts especially methanolic extract of leaves and stem of the plant.

CONCLUSION

All the three extracts (leaves, stem and oil) of *Chrozophora borcchiana* showed dose dependent activity. The leaves methanolic extract was highly active against all bacterial and fungal strains at high dose, while the ethyl acetate extract was active against *Staphylococcus aureus* strain. The stem extract showed medium antimicrobial activity when compared with other extracts. The leaves, stem and oil methanolic extracts of *Chrozophora borcchiana* should further be studied for its phytochemical constituents in order to elucidate the active principle within the extract which can turn out to be a novel antimicrobial agent of the future.

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