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PHYTOCHEMISTRY AND INHIBITORY ACTIVITY OF *CHROZOPHORA SENEGALENSIS* EXTRACTS AGAINST SOME CLINICAL BACTERIAL ISOLATES

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ABSTRACT
Dried leaves of Chrozophora senegalensis were extracted with acetone and hexane respectively using percolation method. The crude leaf extracts were subjected to phytochemical screening for the presence of secondary metabolites using standard procedures. The inhibitory activities of the extracts were tested against clinical bacterial isolates of E. coli, Klebsiella spp., Proteus spp. and Salmonella spp. using disc diffusion and broth dilution techniques. The results of phytochemical screening have demonstrated the presence of alkaloids, reducing sugars, tannins and saponins in acetone extracts while hexane extracts revealed the presence of alkaloids and saponins only. The result of antibacterial activity indicated that all the test isolates were sensitive to hexane extracts while Proteus spp. was the only isolate sensitive to Acetone extracts at equal concentration of 30µg/disc. Apart from Salmonella spp. which had minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 500 and 1000µg/ml, the MIC and MBC of the remaining isolates were greater than the highest concentration 2000µg/ml used in this study.

Keywords: Phytochemistry, Inhibitory activity, Chrozophora senegalensis, Extracts, Clinical bacterial isolates

INTRODUCTION

The use of traditional remedies has relied exclusively on past experiences and observations handed over from generations to generations verbally or in recent times in writing (Sofowora, 1993). The possibilities and potentialities of African medicinal plants as sources for new drugs have not yet been properly researched because less than 10% of the world flora has been studied chemically and in detail. One of the earliest workers in the area of pharmaceutical screening of African Medicinal plants is the team of Finn Sandberg and Panera (Sofowora, 1993).

Various kinds of herbal medicines and treatments available these days provide many advantages for our health. Some of these include the very limited side effects of the same as compared to the benefits of these herbal medicines. These are ideal for those people who are allergic to various kinds of drugs. Most of the times, herbal medicines, can help to treat all kinds of health problems. These include both physical health and mental health of person and these herbal medicines have been used for centuries (Klayman, 1994).

Chrozophora senegalensis is a herb with small green leaves, deep red flowers and violet tingled capsules commonly found dried-up, inundated flats on sandy river bed. The plant belonged to the family Euphorbiaceae and has been reported to serve as remedy for intestinal pain, diarrhea, fever and conjunctivitis (Tignokpa, *et al*., 1986). It is also used in the treatment of intestinal disorders, syphilis, typhoid and boils (Usman *et al*., 2007).

Crude extracts from the leaves and stems of *Chrozophora senegalensis* were found to show the best activity in-vitro against Plasmodium falciparum strains (Tignokpa, *et al*., 1986).

MATERIALS AND METHODS

Collection of plant material

Fresh leaves of *Chrozophora senegalensis* were collected, air dried and grounded into powder using mortar and pestle separately in the laboratory as described by Mukhtar and Tukur (1999).

Extraction

Forty grams each of the dried leaf powder were percolated separately in 400mls each of acetone and hexane in conical flasks stirred well and allowed to stand for 2 weeks with intermittent shaking. The extracts were filtered and concentrated using water bath at 40^{o}C and kept in a refrigerator at 4^{o}C prior to use.

Phytochemical screening

The extracts were subjected to phytochemical tests after filteration before they are concentrated to determine the groups of secondary metabolites present in the plant material.

Test for alkaloids

One milliliter of each of the extract was dispersed in two separates test tubes, 2-3 drops of Dragendoff's and meyer's reagents were separately added. An orange red precipitate/turbidity with Dragendoff's reagent or white precipitate with Meyer's reagent indicated the presence of alkaloids (Ciulci, 1994).

Test for flavonoids

One milliliter of each of the extracts was dispersed in separate test tubes. Sodium hydroxide was added, the appearance of a yellow solution which disappeared on addition of concentrated hydrochloric acid indicates the presence of flavonoids (Oyeleke and Manga, 2008).

Test for saponins

Half gram (0.5g) of the powder of the two plants was taken in separate test tubes to which 5.0ml of distilled water was added and vigorously shaken. A persistent froth that lasted for at least 15 minutes indicated the presence of saponins (Brain and Turner, 1975).

Test for reducing sugars

One milliliter of each fraction of the plants extracts were taken in separate test tubes. These were diluted with 2.0ml of distilled water followed by addition of fehlings solution (A+B) and the mixtures were warmed. Brick-red precipitate at the bottom of the test tubes indicated the presence of reducing sugars (Brain and Turner, 1975).

Test for tannins

Two milliliter of each of the extracts was diluted with distilled water in separate test tubes and 2-3 drops of 5% ferric chloride ($FeCl_3$) solution was added. A green-black or blue black colouration indicated the presence of tannins (Ciulci, 1994).

Confirmatory tests for clinical isolates

The test organisms were clinical isolates obtained from Aminu Kano Teaching Hospital which were further subjected to biochemical tests for re-identification.

a. DCA: The organisms were identified by sub-culture on deocycholate citrate agar (DCA). A black spot was observed which indicate hydrogen sulphide production by *Salmonella.* It was further cultured on simmon citrate agar slant for 24 hours. A deep blue colour was observed which indicated a positive result for Salmonella (Cheesbrough, 2000).
b. EMB: The isolates obtained from the clinic were subcultured on eosine methylene blue agar for 24 to 48 hours. Colonies with green metallic sheen were observed which indicate a positive result for *E. coli*. The colonies were further subjected to urease test which shows a negative result for *E. coli* (Oyeleke and Manga, 2008).
c. Citrate Utilization Test: A colony of the test organisms was cultured on a Simmon's citrate agar slant in a bijou bottle and incubated for 24 to 72 hours. A deep blue colour was observed which indicated a positive result. Most members of the tribe *Klebsiella* and most members of the family *vibrionaceae* are citrate positive. Members of tribe *Escherichia* are distinctly negative while most members of the tribe *Salmonellae* are positive (Oyeleke and Manga, 2008).
d. Urease Test: In this test, a colony of the test organisms was cultured in a urea agar slant in a bijou bottle and incubated for 24hours. The development of a bright pink was observed which indicates a positive result (Oyeleke and Manga, 2008).

The confirmed test organisms were subcultured on a nutrient agar slant in a bijou bottle and kept refrigerated.

Preparation of extract and disc concentrations

The extracts were dissolved using di-methyl sulphoxide (DMSO). Aqueous extract was dissolved using sterile distilled water. 0.002g of the extract was dissolved in 1ml of DMSO as the stock solution. 0.5ml of the stock solution was taken and placed into 50 sterile improvised Whatman No. 1 filter paper discs that take up 0.01ml to make the required disc potency and was labeled 20µg. 0.5ml of DMSO was added into the remaining stock solution making 1ml, 0.5ml was taken and placed into another bottle containing 50 filter paper disc and labelled 10µg/disc 0.5ml of DMSO was added, another 0.5ml was taking and placed into another 50 filter paper disc and labeled 5µg /disc.

Another concentration of the extract was prepared by dissolving 0.1g of the extract in 1ml of DMSO as stock solution. Serial doubling dilution was also carried the same as above and was labeled as 1000, 500 and 250 µg/disc.

Inoculum standardization

Few colonies of the overnight growth of confirmed isolates to be tested were dispensed in sterile normal saline to match the 0.5 McFarland standard for sensitivity tests as described by NCCLS (2000).

Bioassay

The sensitivity testing was achieved by disc diffusion method (NCCLS, 2000). Standardized inocula of the isolates were swabbed onto the surface of prepared and solidified and oven-dried Mueller Hinton Agar in separate Petri-dishes. This was followed by placing four prepared discs onto the surface of inoculated media at intervals in a clockwise direction using augmentin (30µg) as positive control. The plates were incubated at 37^{o}C for 24 hours before observation for and measurement of zones of inhibition formed.

Determination of minimum inhibitory concentration (MIC)

MIC was determined by preparing various concentrations of the extracts by serial doubling dilution and incorporated into test tubes containing 2ml nutrient broth. 0.1ml of standardized inocula of the isolates were introduced and the tubes were incubated at 37^{o}c for 24 hours (NCCLS, 2000).

Determination of minimum bactericidal concentration (MBC)

Nutrient agar plates were inoculated with sample from each of the tubes that show no turbidity and the plates were incubated at 37^{o}c for 24 hours to determine the MBC (NCCLS, 2000).

RESULTS

The results of extraction showed that higher yield of the extracts were obtained on extraction of *Chrozophora senegalensis* using acetone as extraction solvent as shown in Table 1.

Results of phytochemical screening of both plant materials indicated the presence of some secondary metabolites including: alkaloids, reducing sugars, tannins and saponins in acetone extracts while only alkaloids and saponins were detected in hexane extracts as shown in the Table 2.

In-vitro inhibitory activity of the extracts indicated that hexane extract was active against all test organisms while acetone extract was only active against *Proteus spp.* at equal concentrations of 30µg/disc with minimum inhibitory and bactericidal concentrations detected against *Salmonella spp.* as shown in table 3 and Table 4.

Table 1: Some physical Properties of *C. senegalensis extracts*

Properties	AcE	HeE
Weight of powdered plant material (g)	40g	40g
Weight of extract (g)	4.5g	3.5g
Percentage (%) yield	11.25%	8.7%
Colour of extract	Green	Green
Texture of extract	Oily	Oily

Key: AcE - Acetone extract, HeE - Hexane extract

Table 2: Phytochemical Constituents of the extracts

Test	AcE	HeE
Alkaloids (Dragendoff's)	-	+
(Meyers)	+	-
Flavonoids	-	-
Reducing sugars	+	-
Tannins	+	-
Saponins	+	+

Key: AcE – Acetone extract, HeE – Hexane extract

Table 3: Inhibitory activity (mm) of *C. senegalensis* extracts

	AcE(µg/disc)			HeE(µg/disc)			AUG(µg/disc)
Isolates	**15**	**30**	**60**	**15**	**30**	**60**	**30**
E. coli	0	0	0	0	7	8	28
Klebsiella spp	0	0	0	7	7	8	19
Proteus spp	0	7	8	0	7	9	28
Salmonella spp	0	0	0	8	8	9	19

Key: AcE – Acetone extract, HeE – Hexane extract, AUG - Augmentin

Table 4: Inhibitory activity of *C. senegalensis* extracts against the test isolates using micro-broth dilution technique

	AcE (µg/ml)		HeE (µg/ml)	
Isolates	**MIC**	**MBC**	**MIC**	**MBC**
E. coli	**	**	**	**
Klebsiella spp	**	**	**	**
Proteus spp	**	**	**	**
Salmonella spp	**	**	500	1000

Key: AcE - Acetone extract, HeE - Hexane extract, ** - MIC or MBC greater than 1000µg/ml

DISCUSSION

The result of this study indicated that *Chrozophora senegalensis* yielded more extracts when extracted using acetone than using hexane as extraction solvent (Table 1). This showed that acetone extracted more components than hexane from the plant which may be due to difference in polarity of the solvents eventhough the difference is not statistically significant at P = 0.05.

The plant was found to contain some secondary metabolites such as alkaloids, reducing sugars, tannins and saponins when extracted using acetone while hexane extracts revealed the presence of alkaloids and saponins only. This may be due to the fact that hexane solvent used for the extraction was unable to dissolve appreciable amount of the metabolites to be detected by phytochemical screening procedure employed. Also tannins, reducing sugars and alkaloids were not detected in the hexane extract of the two plants. Some of these metabolites particularly the flavonoids were reported to be responsible for antimicrobial activity associated with some ethno-medicinal plants (Usman *et al.*, 2007).

The antibacterial assay showed that acetone extract had no activity on all the isolates except on *Proteus spp.* which showed very slight sensitivity while hexane extract was active against all test isolates but less active than the standard antibiotic disc used at equal concentration of 30µg/disc (Table 3). This may not be unconnected with the fact that the extracts contain both active and a host of inactive compound in their unrefined form.

Hexane extract was very active against *Salmonella spp.* with MIC and MBC values of 500µg/ml and 1000µg/ml respectively while inactive against the remaining isolates even at the highest concentration of 2000µg/ml employed in the study. Also, acetone extract was inactive against all the isolates even at the highest concentration of 2000µg/ml.

The result of sensitivity tests indicated that hexane extract of *Chrozophora senegalensis* was more active against the isolates tested than acetone extract, even-though it contain less number of secondary metabolites than the latter. The activity may be related to the presence of alkaloids and saponins that are well documented for antimicrobial activity (Tschehe, 1971).

CONCLUSION

The results obtained in this study indicated that *Chrozophora senegalensis* has the potential for production of drugs used in the treatment of diarrhea manly caused by *Salmonella specie*.

Recommendations

Further research need to be carried out so as to determine the level of anti-nutrient constituent that may be present in the extracts of the plant. Also Toxicity studies should be carried out using laboratory animals to determine the safety of the plants extracts. The anti-microbial activities of the plants against a wide range of pathogens need to be studied.

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