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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *CYBOPOGON CITRATUS* EXTRACTS AGAINST SOME CLINICAL BACTERIAL ISOLATES

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ABSTRACT
Dried leaf powder of Cybopogon citratus was extracted with distilled water, acetone and hexane using percolation method. The extracts were screened for the presence of alkaloids, flavonoids, reducing sugars, tannins and saponins using standard procedures. Inhibitory activities of the extracts were tested against clinical isolates of Escherichia coli, Salmonella spp., Klebsiella spp. and Proteus spp. using disc diffusion method and broth dilution techniques. The results of phytochemical screening have demonstrated the presence of alkaloids, flavonoids, reducing sugars, tannins and saponins in some or all of the extracts. Similarly, the results of antibacterial activity testing of the extracts at equal disc concentration of 30µg/disc showed that E. coli was sensitive to all extracts with inhibition zone diameters of 7mm, Klebsiella spp. and Proteus spp. were sensitive to acetone extract of the plant with inhibition zone diameters of 7mm each while Salmonella spp. was sensitive to acetone and hexane extracts of the plant with inhibition zone diameters of 7mm and 8mm respectively. Salmonella spp. demonstrated MIC and MBC values of 1000µg/ml and 2000µg/ml respectively. These results suggest that C. citratus used in this study has the potential for the production of drugs against bacterial infections.

Keywords: Phytochemical properties, Antibacterial activity, Cybopogon citratus, Extracts, Bacterial isolates

INTRODUCTION

Herbal remedies have the capacity to bring a certain amount of effect in the body and prove to be effective in treating some health problems. This result in several pharmaceutical companies being engaged in the development of natural product drugs through the isolation of active molecules from plant extracts which results in the provision of models for over 50% of Western drugs (Janny, 2009).

Cybopogon citratus is a tall aromatic coarse grass belonging to the family poaceae. It is a monocotyledonous hypogeal perennial plant with slender sharp edged green leaves that have pointed apex. The stem is reddish brown in colour and is attached to the bulb by stalk with the entire plant being attached to the soil by fibrous root. A tea made from the leaves of C. citrates is used in the treatment of fevers, cough, stomach upset and urinary tract infections (Shadab *et al*, 1992).

Lemon grass is commonly used as teas, soaps and curries suitable for poultry, fish as well as sea food (Shadab *et al*, 1992). It is also used as pesticide and a preservative study indicated that its oil has antifungal properties (Hammer *et al*, 1999). A tea made from *C. citratus* leaves has been used locally to treat typhoid fever, cold, cough and stomach upset (Shadab *et al*, 1992). It has been found to cause programmed cell death (apoptosis) in cancer cells and the oil is found to be active against human dermatophytes (Hammer *et al*, 1999). The oil has been reported to have the potential for new and safe agents for inclusion in anti-*Helicobacter pylori* regimens (Tohno *et al*, 2003).

This study was designed to determine the phytochemical constituents and the antibacterial activity of different *Cybopogon citratus* leaf extracts against some clinical bacterial isolates.

MATERIALS AND METHODS

Collection and identification of Plant Materials

The leaves the plant was hand-picked and identified by Professor B. S. Aliyu of Biological Sciences Department, Bayero University, Kano. It was air-dried under shade and ground in to powder using mortar and pestle and then sieved as described by Mukhtar and Tukur (1999).

Aqueous Extraction

Thirty grams of powered plant leaves was steeped in 300ml of distilled water and left for two weeks with regular shaking, after which they were then filtered using whatman No.1 filter paper. The extracts were concentrated using water bath at 80^{o}C (Fatope *et al*, 1993).

Organic Solvent Extraction

Thirty grams of each powdered plant material was soaked in 300ml of each of acetone and hexane in separate conical flasks and allowed to stand for 2 weeks at room temperature with persistent shaking, after which they were filtered using filter paper.

The filtrate obtained were then concentrated in vacuo using rotary evaporator (R114) machine at 4°C (Fatope *et al*, 1993).

Phytochemical Test

Test for Alkaloids

Two to three drops of Dragendoff's and Meyer's reagent were separately added to 1.0ml of each extract in two separate test tubes. An orange red precipitate with Dragendoff's reagent and white precipitate with Meyer's reagent indicated the presence of alkaloids (Ciulci, 1994).

Test for Saponins

Five mls of distilled water was added to 0.5g of the powder in a test tube and shaked vigorously. A persistent froth that lasted for 15 minutes indicated the presence of saponins (Brain and Turner, 1975).

Test for Tannins

Two mls of each of the extract 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).

Test for Flavonoids

One ml of each extract was dissolved in sodium hydroxide solution. The appearance of a yellow solution which disappeared on addition of hydrochloric acid indicates the presence of flavonoids (Oyeleke and Manga, 2008).

Test for Reducing Sugar

Two mls of distilled water was used to dilute 1ml of each of the extracts contained in separate test tubes. This was followed by addition of Fehling's solution (A+B) and the mixtures warmed. Brick red precipitate at the bottom of the test tubes indicated the presence of reducing Sugar (Brain and Turner, 1975).

Biochemical Identification of the Test Organisms

The test organisms were clinical isolates obtained from Aminu Kano Teaching Hospital and werereconfirmed using routine bacteriological and biochemical tests including indole, motility, citrate utilization, urease production, hydrogen sulfide production as well as acid and gas production for identification according to standard procedures (Cheesebrough, 2005; Oyeleke and Manga, 2008).

Preparation of Sensitivity Discs

Whatman No.1 filter paper discs (of 6mm in diameter) were punched out with the aid of perforator and placed in Bijour bottles. They were then sterilized by autoclaving at 121°C foe 15minutes. The discs were allowed to cool (Mukhtar and Tukur, 1999).

Preparation of Stock Solution

Sixty milligrams of the extracts were dissolved in 1ml dimethyl sulphoxide (DMSO) while aqueous extracts were dissolved in 1ml sterile distilled water for water extract and DMSO for other extracts. Half (0.5) ml of the extract was introduced into 50 sterile discs respectively in Bijour bottles to make 60µg/disc concentration. Half ml of DMSO was added into the remaining stock solution making 1ml, 0.5 ml was taken and placed into another bottle containing 50 filter paper discs and labeled 30µg/disc, 0.5 ml of DMSO was added, another 0.5 ml was taking and placed into another 50 filter paper discs and labeled 15µg/disc. The same process of serial doubling dilution as explained above was employed in the preparation of organic solvent extract discs.

Standardization of Inoculum

Using inoculation loop, a loopful of colony from a 24 hour culture of the test organism was transferred into a test tube containing normal saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard as described by the National Committee for Clinical Laboratory Standard (2008).

Bioassay Procedure

Standard inocula of the isolate were swabbed on to the surface of prepared and solidified Mueller Hinton agar in separate Petri-dishes. The prepared discs of the extracts and the standard antibiotic discs (Augmentin) were placed onto the surface of the inoculated medium at intervals. The plates were incubated at 37°C for 24 hours before observation for and measurement of zones of inhibition (NCCLS, 2008).

Minimum Inhibitory Concentration

MIC was determined by preparing various concentrations of the extracts by serial doubling dilutions and incorporated into test tubes containing 2ml nutrient broth followed by introduction of 0.1ml of standardized inocula of the isolates and the tubes were incubated at 37°c for 24 hours. Tubes containing broth and plant extracts without inocula served as positive control while tubes containing broth and inocula served as negative control. The set of tubes were incubated aerobically at 35°C for 24 hours after which the lowest concentration that showed no evidence of growth was recorded as the MIC (NCCLS, 2008).

Minimum Bactericidal Concentration

Nutrient agar plates were inoculated with samples from each of the tubes that showed no turbidity and the plates were incubated at 37°C for 24 hours to determine the MBC. The highest dilution that yielded no bacterial colony was taken as the MBC (NCCLS, 2008).

RESULTS

The results of extraction showed that higher yield of the extracts were obtained in *C. citratus* aqueous extracts which was green in colour and oily in texture (Table 1).

Results of phytochemical screening of both aqueous and other extracts indicated the presence of alkaloids, reducing sugars, saponins, tannins and flavonoids (Table 2).

In-vitro inhibitory activity of the extracts indicated that all the extracts were active against the test organisms when compared to the inhibition zones formed in response to the standard antibiotic (Augmentin) used as control at equal concentrations of 30µg/disc (Table 3).
Sensitivity test using broth dilution method showed that acetone extract was the only extract active and only against *Salmonella* spp. with MIC and MBC values of 1000µg/ml and 2000µg/ml respectively.

Table 1: Physical Properties of *C. citratus* extracts

Properties	AcE	HeE	AqE
Weight used for extraction (g)	30	30	30
Weight of extract (g)	3.9	3.0	4.6
Percentage yield (%)	13	10	15.3
Colour	Green	Green	Green
Texture	Oily	Oily	Oily

Key: AcE - Acetone extract, HeE - Hexane extract, AqE - Aqueous extract

Table 2: Phytochemical constituents of *C. citratus* extracts

Test	AcE	HeE	AqE
Alkaloid(Dragendoff's)	+	-	+
(Meyers)	+	+	-
Flavonoid	-	-	+
Reducing Sugars	+	-	+
Tannins	+	-	+
Saponins	+	+	+

Key: AcE - Acetone extract, HeE - Hexane extract, AqE - Aqueous extract, + = Present, - = Absent

Table 3: Inhibitory activity of *C. citrates* extracts (µg/disc) against the test isolates

Isolates	AcE (µg)			HeE (µg)			AqE(µg)			Standard
	15	30	60	15	30	60	15	30	60	AUG (30µg)
E. coli	7	7	7	6	7	8	7	7	7	33
Klebsiella spp.	6	7	8	6	6	6	6	6	6	23
Proteus spp.	6	7	9	6	6	6	6	6	6	16
Salmonella spp.	7	7	8	7	8	8	6	6	6	19

Key: AqE - Aqueous extract, HeE - Hexane extract, AcE - Acetone extract

Table 4: Inhibitory activity of *C. citratus* extracts (µg/ml)against the test isolates using micro-broth dilution technique

Isolates	AqE(µg/ml)		AcE (µg/ml)		HE (µg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	**	**	**	**	**	**
Klebsiella spp.	**	**	**	**	**	**
Proteus spp.	**	**	**	**	**	**
Salmonella spp.	**	**	1000	2000	**	**

Key: AqE - Aqueous extract, AcE - Acetone extract, HeE - Hexane extract, ** **-** MIC or MBC greater than 2000 µg/ml

DISCUSSION

The findings of this study showed that *Cybopogon citratus* yielded more extract when subjected to water extraction with oily texture and green appearance. This showed that water has a stronger extraction capacity than acetone and hexane. This may be related to high polarity of most of the compounds contained in the plant extract.
The plant was found to contain some secondary metabolites including; alkaloids, reducing sugars, saponins tannins and flavonoids even though the flavonoids were only present in aqueous extract. This may be due to the fact that some solvents used in the extraction were unable to dissolve appreciable certain amount of the metabolite to be detected by phytochemical screening procedure employed. Reducing sugars, flavooids and tannins were not detected in hexane extract of the plant. Some of these metabolites particularly the flavonoids were reported to be responsible for antimicrobial activity associated with some ethnomedicinal plants (Singh and Bhat, 2003).
The antimicrobial assay using disc diffusion method showed that all the extracts were slightly active gainst *E. coli* at concentration of 30µg/disc while only acetone extract showed promising activity against *Salmonella* spp. when tested using broth dilution method despite the absence of flavonoids in the extracts. This suggested that the activity could be related to the presence of alkaloids and tannins that are well documented for antimicrobial activity (Tschehe, 1971).

Conclusion and Recommendations

The demonstration of activity of *C. citratus* extracts against different groups of pathogenic bacteria is a scientific justification of the local application of the plant as a health remedy. Further research needs to be carried out in order to isolate and identify the active compound(s) present in the plant extract. Toxicity studies should also be carried out to determine the safety of the plant extracts/active compounds.

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