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Full Length Research Paper

A study on antimicrobial effect of extracts of *Cassia arereh* (Del.) on some clinical isolates

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Results of preliminary phytochemical analysis showed that the stem and root barks of *Cassia arereh* (Del.) possess phenols, tannins, cardiac glycosides, phlobatanin and anthraquinone. The acetone extract of the root bark produced the highest antimicrobial activity against *Escherichia coli* (16 mm), *Klebsiella pneumoniae* (11 mm) and *Staphylococcus aureus* (9 mm) compared to the other extracts. All the extracts did not possess significant antimicrobial activity against *Salmonella typhi, Streptococcus pyogenes* and *Candida albicans* at 100 mg/ml concentration. The aqueous extracts of the root and stem barks did not produce any measurable antimicrobial activity against all the tested organisms. Warming to 60°C increased the activity of the root bark extract especially the acetone extract (11 - 36%) against *E. coli, K. pneumoniae* and *S. aureus* while in the case of the stem bark, the activity increased at 11% for these organisms. The MIC values for the acetone extract of the root bark were in the range of 25 – 50 mg/ml whereas the MBC values were 100 mg/ml against the susceptible organisms. So the use of *C. arereh* for the treatment of boils, wound infections, urinary tract infections, respiratory tract infections, diarrhoea and scarlet fever may be justified.

Key words: Phytochemical, MIC, MBC, micronization, *Cassia arereh*.

INTRODUCTION

Antimicrobial substances are substances that inhibit the growth and existence of microorganisms (Paul and Sainburg, 1994). These micro organisms could be pathogenic or non pathogenic, hence, antimicrobial substances are used in the treatment of various ailments. Quite a number of antimicrobial substances exist and they are gotten from diverse sources such as microbial, plant, animal and chemical sources (Ganellin and Roberts, 1999). Medicinal uses of these plants range from the administration of the plant's roots, bark, stem, leaves, fruits and seeds, to the use of extracts from the whole plant (Akujobi et al., 2004). Plants have a great potential for producing new drugs of great benefit to mankind. There are many approaches to the search for new biologically active principles in higher plants (Jigna and Chanda, 2006). This search for new antimicrobial properties of natural products cannot be ignored because this can be found in the most remote parts of the world where medical doctors are not present (Oukemi and Kandakai, 2004). Among the diseases that have been managed successfully by traditional (herbal) medicine include malaria, epilepsy, infertility, convulsion, diarrhoea, dysentery, gonorrhoea, flatulence, tonsillitis, bacterial and fungal infections, mental illness and worm infections (Sofowora, 1996).

Natural selection during evolution and competition between organisms has produced powerful biologically active natural products which can serve as chemicals and have been refined by modern techniques to give more specifically active drugs (Ganelline and Robert, 1999). Garlic cloves (*Allium sativum*) are used as a remedy for infections and digestive disorders (Groppo et al., 2007; Shufford et al., 2005). Garlic has been reasonably successfully used in AIDS patients to treat cryptosporidium in an uncontrolled study in China. It has also been used by AIDS patients to treat toxoplasmosis, a protozoan disease (James, 1988). The ethanolic extract of *Launaea procumbens* (Roxb) was active against more than 70% of microorganisms investigated. The ethanolic as well as the aqueous extract of *Vitris vinifera* L. was active against more than 85 and 65% of the studied bacterial strains respectively (Jigna and Chanda, 2006).

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A recent study showed that henna leaf extracts were capable of inhibiting the growth of bacterial species such as *S. aureus, Streptococcus sp., Pseudomonas aeruginosa* and fungal species such as *Aspergillus niger* and *Fusarium oxysporum* (Muhammad and Muhammad, 2005).

Cassia arereh is a small tree which belongs to the family Caesalpiniaceae. It is found in the Sudanese savannah, on shallow but quite rich soil. It is found also in northern Nigeria, Cameroun, Ethopia and Eritrea. It is used in Nigerian traditional medicine for the treatment of diarrhoea, dysentery, dermatitis, malaria and skin infections.

In view of the fact that there are no published information regarding the use of *C. arereh* as an antimicrobial agent, this study is designed to evaluate whether *C. arereh* have some active principles that could be used for chemotherapeutic purposes.

MATERIALS AND METHODS

Collection and identification of the plants

The materials used were stem and root barks of *C. arereh* and these were collected in mid-April beforethe first rain from Mulduw area about 2 km away from Gashala village in Hong local government area of Adamawa State, Nigeria and were brought to the Department of Biological Sciences, Federal University of Technology, Yola for identification purposes. The materials were identified and authenticated by Mr. Bristone Basiri of the Department of Biological Sciences, Federal University of Technology, Yola (FUTY).

Drying and micronization of the samples

The root and stem barks of *C. arereh* were cut into small pieces using a sharp knife and then dried in the laboratory under room temperature for 7 days. The dried samples were then crushed using mortar and pestle; later the crushed materials were further ground to powder form with an electric blender (National model No. Mx-795-1 L). This was done to enhance the penetration of the extracting solvents into the cells, thus, facilitating the release of active principles (Jigna and Chanda, 2006). The ground samples were then used for extraction purposes.

Preparation of the extracts from micronized samples

Ethanol, acetone and water were employed as solvents for the extraction of both samples (root and stem barks of *C. arereh*). Herbalists generally use the herbs with water and not ethanol because of its broad spectrum and relative non-selection of extracting tannins (Jigna and Chanda, 2006).

Aqueous extracts

About 100 ml of distilled water was added to 25 g each of the two powdered samples (root and stem barks) of *C. arereh* in a 250 ml conical flask and was then soaked for 2 h. After that period, the flask was agitated manually, after every 30 min for 3 h. The mixture was then allowed to settle down and left overnight in the refrigerator. In each case, the extract was initially filtered using a clean muslin cloth and then by using a Whatman's No. 1 filter paper. A rotary evaporator was used in vacuum at 40°C to concentrate each of the extracts (De and Nanja, 2001). The dried extracts obtained were stored in the refrigerator at 4°C for further use.

Ethanol extracts

About 25 g each of the powdered samples was dispensed into a 250 ml conical flask and 100 ml of 97% ethanol was added to the sample. The procedure was similar to that of aqueous extracts except that ethanol was used instead of water; the recovered extracts were kept in the refrigerator for further use.

Acetone extracts

The procedure was similar to ethanol extracts except that acetone was used instead. The recovered extracts obtained from both the root and stem barks of *C. arereh* were stored in the refrigerator for further use.

Preliminary phytochemical screening of stem and root barks of *C. arereh*

A preliminary phytochemical analysis to screen the samples for the presence of phytochemical components such as alkaloids, anthraquinone, cardiac glycosides, phlobatanins, phenol, saponins and tannins was performed according to the method described by Grand and Wondergem (1985).

Source of microorganisms

The organisms were collected from the Microbiology Department of the Specialist Hospital, Yola. The bacterial species include *E. coli, S. aureus, S. pyogenes, S. typhi, K. pneumoniae* and *C. albicans*

Determination of antimicrobial activity using disc diffusion method

Each dried acetone and ethanol extracts of the root and stem barks of *C. arereh* was reconstituted with ethanol to get a final concentration of 100 mg/ml which was used to impregnate the discs for susceptibility testing. Equally the aqueous extracts were reconstituted using distilled water to get a final concentration of 100 mg/ml.

The disc diffusion was used according to Duraipandiyan et al. (2006). *In vitro* antimicrobial activity was screened by using the Mueller Hinton agar for the bacteria species and the Sabouraud dextrose agar for *C. albicans*. Antimicrobial activity was evaluated by measuring the diameter of zone of inhibition of the extract. Antimicrobial activity was expressed as the diameter of zone of inhibition calculated as the difference between the diameters of the observed zone and the disc (6 mm). Standard antibiotic disks comprising penicillin (50 µg), tetracycline (50 µg), amoxicillin (25 µg), cortimoxazole (50 µg), nitrofurantoin (100 µg) gentamycin (10 µg), nalidixic acid (30 µg), ofloxacin (30 µg and augmentin (30 µg) were used against some test organisms for comparison purposes . For *C. albicans*, ketoconazole was used at 0.75 mg/ml concentration. Ofloxacin produced significant antibacterial activity against *E. coli*, *S. aureus*, *S.typhi* and *K. pneumoniae (*11 - 20 mm*)*. Ketoconazole produced significant antifungal activity against *C. albicans* (8 - 10 mm).

Table 1. Characteristics of extracts obtained from root and stem barks of *C. arereh* (Del.).

Extracts	pH	Amount (100 g of sample) g	Colour	Consistency
RACE	4.91	14.8	Dark brown	Solid
RETE	4.82	17.2	"	"
RAQE	5.20	10.0	Brownish	Semi solid
SACE	4.03	11.6	"	Solid
SETE	4.2	13.6	"	"
SAQE	5.40	9.2	"	Semi solid

Key: RACE – root bark acetone extract; RETE – root bark ethanolic extract; RAQE - root bark aqueous extract; SACE – stem bark acetone extract; SETE - stem bark ethanolic extract; SAQE - stem bark aqueous extract.

Table 2. Premilinary phytochemical screening of samples.

Components	Root bark	Stem bark
Alkaloids	-	-
Anthraquinone	+	+
Cardiac glycosides	+	+
Phlobatanins	+	+
Phenols	+	+
Saponins	-	-
Tanins	+	+

Key: + = present; - = absent.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extracts

Acetone extract of the root bark which showed significant antimicrobial activity was used for this purpose. Antimicrobial activity was evaluated by measuring the diameter of zone of inhibition of the extract. Antimicrobial activity was expressed as the diameter of zone of inhibition calculated as the differences in the diameters of the observed zone and the disc (6 mm). MIC and MBC of the extract was determined using *S. aureus, K. pneumonia and E.coli* (Jigna and Chanda, 2006).

RESULTS

Physiochemical characteristics of extracts

Preliminary phytochemical screening: The result of preliminary phytochemical analysis (Table 1) shows that the extracts from both plant parts possess anthraquinone, cardiac glycosides, phlobatanins, phenol and tannins.

Phytochemical activity of the extracts

Phytochemical activity of the extracts is shown in Table 2

Antimicrobial activity of extracts

The acetone and ethanol extracts of the root and stem barks for *C. arereh* showed the highest antimicrobial activity against *E. coli*(16 mm), followed by *K. pnuemoniae* (11 mm), *S. aureus* (8 mm) and *S. typhi* (2 mm). Antimicrobial activity was expressed as the diameter of zone of inhibition calculated as the differences in diameters between the observed zone and the disc (6 mm). The extracts did not possess any measurable zone of inhibition against *C. albicans* and *S. pyogenes* at 100 mg/ml. Aqueous extracts of both root and stem barks did not show measurable zone of inhibition against the tested organisms at this concentration. The results are shown in Table 3.

Determination of minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) of extracts

The MIC and MBC values (mg/ml) of the extracts are shown in Table 4.

DISCUSSION AND CONCLUSION

Results of phytochemical screening indicates that the root and stem barks of *C. arereh* contain active chemical components like anthraquinone, cardiac glycosides, phlobatanins, phenols and tannins. The antibacterial activity of these extracts may be due to the presence of these active components. Clark (1981) reported that the root and stem barks of *Magnolia olifera* contain two alkaloids and their extracts inhibit the growth of certain bacterial and fungi. Also Okwute (1992) observed that saponins, phenolic compounds and cardiac glycosides are known to possess definite antimicrobial and pesticide properties. Antimicrobial activity test revealed that the acetone extract of the root bark possess the highest antimicrobial activity against *E. coli* (16 mm), followed by *K. pnuemoniae* (11 mm), and *S. aureus* (8 mm) when compared to the ethanol extract (*E. coli*- 11 mm, *K. Pnuemoniae*- 9 mm and *S. aureus*- 6mm). Acetone is known to extract phytochemical components like tannins while ethanol extracts some phytochemicals like tannins and polyphenols. The high antimicrobial activity of the acetone extract

Table 3. Antimicrobial activity of extracts (zone of inhibition in mm) against selected microorganisms.

Microorganisms	Root bark			Stem bark		
	ACE	ETE	AQE	ACE	ETE	AQE
S. pyogenes	N	N	N	N	N	N
S. aureus	8	6	N	7	4	N
E. coli	16	11	N	9	7	N
S. typhi	2	N	N	N	N	N
K. pnuemoniae	11	9	N	8	7	N
C. albicans	N	N	N	N	N	N

Key: ACE = acetone extract; ETE = ethanol extract; AQE = aqueous extract; N = negligible.

Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of acetone extract of the root bark of *C. arereh.*

Microorganisms	MIC (mg/ml)	MBC (mg/ml)
E. coli	25	100
S. aureus	50	100
K. pnuemoniae	50	100

of the root bark may be due to the extraction of higher amounts of phytochemicals compared to that of ethanol. The acetone extract of the stem bark (*E. coli* – 9 mm, K. *pneumoniae* – 7 mm and *S. aureus* – 6mm) produced lower activity compared to that of the ethanol extract (*E. coli* – 7 mm, *K. pnuemoniae* – 6mm and *S. aureus* – 4 mm). It was also observed that the root bark extract produced higher activity compared to the stem bark extract.

This study demonstrated that the activity of the extracts depend on the solvent employed in their extraction from the plant samples, while the root extracts indicated strong antibacterial effects compared to the stem. This indicates that the phytochemical constituents are more active in the root than in the stem.

In conclusion, this study has shown that extracts from *C. arereh* possess antimicrobial pro-perties thus justifying its use in traditional medicine. From the experiment carried out, the use of *C. arereh* for the treatment of boils, wound, urinary tract and respiratory tract infections, diarrhoea and scarlet fever is justified. It has also been shown that extracts from the root bark were more effective against *E. coli, S. aureus* and *K. pnuemoniae* than extracts from the stem bark.

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