

Full Length Research Paper

Phytochemical and antimicrobial screening of the aqueous extract of *Cassia arereh* Del. stem-bark

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The phytochemical constituents and antimicrobial properties of the aqueous extract of *Cassia arereh* stem-bark were evaluated. The extract was subjected to qualitative chemical screening of active chemical constituents and disc diffusion method was performed to determine the antimicrobial properties. The results revealed the presence of tannins, phlobatannins, carbohydrate, saponin, flavonoids, terpenes and steroids while anthraquinones, alkaloids and cardiac glycosides were not detected. The extracts (100, 200, 400 and 800 mg/ml) inhibited the growth of *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Pseudomonas aerogenosa* and *Enterobacter* species. However, *Escherichia coli*, *Klebsiella pneumoniae*, *Corynebacterium pyogenes* and *Candida albicans* were resistant. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values were 50 mg/ml for *S. aureus* and *B. subtilis*, and 100 mg/ml for *P. aerogenosa*. It was concluded that the aqueous extract of *C. arereh* stem-bark contained active principles having antibacterial properties and thus support the folkloric use of the plant.

Key words: Phytochemical, aqueous extract, antimicrobial, *Cassia arereh*.

INTRODUCTION

Traditional medicine is undoubtedly the oldest form of medicine and probably evolved simultaneously with the evolution of human beings (Wynn, 2001; Wanzala et al., 2005) or even much earlier. Its ability to stand the test of time over the millennia to its current situation could not be unconnected to its important contribution to the maintenance of health. In fact the primary health care of about 80% of the world's population is dependent on the use of medicinal plants derived from traditional medicine (Bajaj and Williams, 1995). The World Health Organization (WHO) states that 74% of these plants have modern indications that correlate with their traditional, cultural and sometimes ancient uses (Wynn, 2001). In Africa more people seek medical attention from traditional medical practitioners than from medical doctors.

Medicinal plants serve as the main source of medicine to rural poor communities that do not have access to modern medical services.

About 25% of conventional drugs are derived from plants that are been used traditionally (Spore, 1992). Investigations into the antimicrobial activity of some medicinal plants are been carried out (Kudi et al., 1999; Ogundipe et al., 2000; Isaac and Chinwe, 2001; Geidam et al., 2007; Sanni, 2007) with a view to authenticate their folkloric use.

The plant *Cassia arereh* Del. is a wild small tree, 2 - 5 m high that belongs to the family Caesalpinioideae. It is locally called Marga, Maleduwa, Mihuski or Dandarazo in Hausa; Cabbu or Jutihi in Fulfulde; Mihuski in Gwari; kurnggilang in Babur-Bura and Maraguwa in Kare-kare languages (Gambo and Karofi, 2004; Blench, 2009). Almost all parts of the plant are used locally as medicine. The root and the stem-bark are used in disease conditions such as diarrhoea, dysentery, stomach ache, ascites, headache, cough, rheumatism, back pain, wound healing, weakness, avian plague, yellow fever and malaria. The fruit pulp is used as laxative while the leaves

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are used as diuretic, antipyretic, analgesic and in the treatment of pleurisy and burns. The seed is used for treatment of pneumonia and for magico-religious purposes (Arbonnier, 2004). However little scientific information is available on this plant. This study investigated the phytochemical constituents and *in vitro* antimicrobial properties of the plant.

MATERIALS AND METHODS

Plant collection, identification and extract preparation

Fresh leaves, fruits and stem-bark of *C. arereh* were collected in August, 2008 from Ngulde district in Borno State, Nigeria, and were confirmed and authenticated by Prof. S. S. Sanusi of the Department of Biological Science, University of Maiduguri, Nigeria. The stem-bark was air dried at room temperature for two weeks and thereafter was pulverised into fine powder. Two hundred grams (200 g) of the powder was mixed with 1.2 L of distilled water (25°C) in a beaker and the mixture was shaken vigorously. Shaking was repeated after every 30 min for the next 6 h before it was allowed to stand for 18 h. Thereafter it was shaken vigorously before it was filtered using Whatmann No. 1 filter paper. The filtrate was dried in an oven (DHG-9030A) at 50°C and stored in a glass container at 4°C until required.

Phytochemical screening of the aqueous extract of *Cassia arereh* Stem-bark

The crude aqueous extract of *C. arereh* Del. was subjected to qualitative chemical screening for the identification of the various classes of active chemical constituents. The phytochemical analyses were carried out according to standard methods described by Trease and Evans (1997) and Sowofora (2006).

Microbial Cultures

Laboratory isolates of *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Pseudomonas aerogenosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Corynebacterium pyogenes*, *Enterobacter* species and *Candida albicans* were obtained from the Department of Veterinary Medicine laboratory, University of Maiduguri, Nigeria. The isolates were cultured separately on nutrient agar plate and incubated for 24 h. The medium (25 ml) was poured into sterile Petri dishes and allowed to solidify. A colony of each test organism was sub cultured on 10 ml nutrient broth and incubated at 37°C for 8 h. One millilitre of the broth culture was used to flood the agar plates.

Antimicrobial activity

The disc diffusion method as described by National Committee for Clinical Laboratory Standards (NCCLS, 1993) was used to determine the growth inhibition of bacteria by the plant extract. Discs containing different concentrations (100, 200, 400 and 800 mg/ml) of dissolved extract were prepared using sterile Whatmann filter paper No. 1, (6 mm in diameters). The discs were dried at 50°C. Over-night cultures of each of the bacterial isolates was diluted with sterile normal saline to give inoculum size of 10^6 cfu/ml. Nutrient agar medium was prepared, sterilized, cooled and poured into sterile Petri dishes to a depth of 4 mm (about 25 ml per plate) to solidify. Pure cultures of the test organisms were used to inocu-

late the Petri dishes. This was done by spreading the inocula on the surface of the prepared nutrient agar plate using sterile cotton swabs which have been dipped in the diluted suspension of the organism. The discs were then aseptically placed evenly on the surface of the inoculation and gently pressed down to ensure contact using a pair of forceps. The plates were finally incubated at 37°C for 24 h. Tetracycline (25 mg/ml) (Greenfield Pharmaceutical Co. LTD, JIANGSU Province, China) was used as positive control in each plate. Plates prepared using the same procedures without extract or antibiotic were equally set as negative control. The plates were examined after 24 h for clear zone of inhibition. Antibacterial activity by the extract was measured using a transparent ruler and recorded as the difference in diameter between the clear zone and the disc (6 mm).

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The method of Greenwood (1989) was used to determine the M.I.C and M.B.C of the extract. Five sterile test tubes were arranged in a test tube rack and 0.5 ml of sterile nutrient broth was pipetted into each test tube. Half ml of the crude extract containing 200 mg/ml was pipetted into each of the five tubes containing the broth. Thereafter, there was a serial dilution of the extract to obtain concentrations of 100, 50, 25, and 12.5 mg/ml respectively. The test organism (0.5 ml) was pipetted into each of the test tubes containing the mixture of the broth and extract and then finally incubated at 37°C for 24 h. The MIC was recorded as the least concentration of plant extract that completely inhibited the growth of the test organism. The MBC was determined by sub culturing the contents of the tubes for 24 h to determine bactericidal activity. Bactericidal effect was demonstrated when no growth occurred on the sub cultured medium after M.I.C. determination.

Statistical analysis

All data generated during the course of the research were expressed as mean \pm standard deviation (S.D.) and analysed statistically by analysis of variance (ANOVA).

Significant difference was considered at $P \leq 0.05$ using Tukey post test. Graph pad InStat (2000) computer statistical software package was used for the analysis.

RESULTS

The result (Table 1) of the phytochemical screening showed that tannins, phlobatannins, saponins, flavonoids, terpenes, steroids and carbohydrates were present while anthraquinones, alkaloids and cardiac glycosides were absent in the aqueous extract of *C. arereh* stem-bark. The antimicrobial activity of the extract is presented in Table 2, and the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) are presented in Table 3. The extract produced dose-dependent which were less effective than tetracycline, zone of inhibition in *S. aureus*, *B. subtilis*, *S. typhi* and *P. aeruginosa*. At 800 mg/ml the inhibition were, respectively, 8, 9, 6 and 6 mm as well as 4 mm for *Enterobacter species*. However, *E. coli*, *K. pneumoniae*, *C. pyogene* and *C. albicans* were resistant. The MIC and MBC was 50 mg/ml for *S. aureus* and *B. subtilis*, and 100 mg/ml for

Table 1. Phytochemical screening of aqueous extract of *C. arereh* stem-bark.

Phytochemical constituent	Test	Inference
Carbohydrate	Molisch's	+
	Barfoed's	-
	Fehling's	+
	Combined reducing sugar	+
	Ketones	+
	Pentoses	+
Tannins	Ferric chloride	+
	Formaldehyde	-
	Chlorogenic	-
Phlobatannins	Hydrochloric acid	+
	Lime water	-
Alkaloids	Mayer's	-
	Dragendorff's	-
Anthraquinones	Free anthraquinones	-
	Combined anthraquinones	-
Saponin	Froth	+
Cardiac glycoside	General test	-
Terpenes and Steroids	Lieberman Burchard	+
	Salkowski	+
Flavonoids	Lead acetate	+
	Sodium hydroxide	+
	Ferric chloride	+
	Pew's	+

- = present; - = not detected.

Table 2. Antimicrobial properties of aqueous extract of *C. arereh* stem-bark.

Dosage (mg/ml)	Zone of inhibition (mm)								
	St	Ba	Sa	Ps	En	Es	KI	Co	Ca
100	3	3	R	2	R	R	R	R	R
200	4	5	R	4	R	R	R	R	R
400	7	7	5	4	R	R	R	R	R
800	8	9	6	6	4	R	R	R	R
Control	10	22	21	15	24	11	3	24	30

St = *Staphylococcus aureus*, Ba = *Bacillus subtilis*, Sa = *Salmonella typhi*, Ps = *Pseudomonas aerogenosa*, En = Enterobacter, Es = *Escherichia coli*, KI = *Klebsiella pneumoniae*, Co = *Corynebacterium pyogenes*, Ca = *Candida albicans*, R = Resistant, Control = tetracycline 250 mg/ml.

Table 3. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of aqueous extract of *Cassia arereh* stem-bark.

Organism	MIC (mg/ml)	MBC (mg/ml)
<i>Staphylococcus</i> spp.	50	50
<i>Bacillus</i> spp.	50	50
<i>Pseudomonas</i>	100	100

P. aeruginosa.

DISCUSSION

The phytochemical screening of aqueous extract of *C. arereh* stem- bark revealed the presence of important chemical constituents such as tannins, phlobatannins, saponins, flavonoids, terpenes, steroids and carbohydrates. Screening conducted on the plant obtained in Adamawa State, Nigeria (De et al., 2009) indicates the presence of anthraquinones, cardiac glycosides, phlobatanins, tannins and phenols. Variations of chemical constituents from this study are possible because of difference of geographical location. Tannins have been reported for its astringent properties which hasten wound healing and ameliorate inflamed mucous membrane (Mota et al., 1985; Tyler et al., 1988). Saponins may have expectorant (Finar, 1989) or antidiarrheal (Al-Rehaily et al., 2001) properties. Flavonoids have free radical scavenging actions and possess antimicrobial (Narayana et al., 2001), anti inflammatory (Middleton et al., 2000), antioxidant (Parker et al., 1999) and anti tumour (Inoue and Jackson, 1999) activities. Steroids have anti-nociceptive properties (Miguel et al., 1996).

This study also indicates that the aqueous extract of *C. arereh* stem bark at the concentration of 800 mg/ml possessed *in vitro* antibacterial activities against *S. aureus* (8 mm), *B. subtilis* (9 mm), *S. typhi* (6 mm), *P. aeruginosa* (6 mm) and *Enterobacter species* (4mm). This is in contrast to the work of De et al. (2009) which reported that the aqueous extract of *C. arereh* (100 mg/ml) sourced in Adamawa State, Nigeria did not possess any antibacterial activities against organisms tested. The work also reported that acetone and ethanol extracts produced antibacterial activities. This variation could be due to geographical location of the plant and concentrations used. The antibacterial activity could be attributed to one or more of the chemical constituents present in the extract particularly flavonoids and tannins (Robinson, 1967; Narayana et al., 2001; Sani et al., 2009). The activity in each organism was however lower than that of tetracycline 25 mg/ml. There was no activity in *C. albicans* and this may suggest that the extract lack antimycotic properties.

Conclusion

This study showed that the plant contains active principles that may be responsible for the antibacterial properties observed and thus supports the traditional medical use of the plant. However further study needs to be carried out to identify the key active principles.

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