

Full Length Research Paper

Elemental analysis and Anti-microbial potentials of the leaf extract of *Cassia arereh* Del.

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Accepted 1 November, 2011

The phytochemical analysis, elemental analysis, antimicrobial activities and the minimum inhibitory concentration (MIC) of aqueous and ethanolic extracts of the leaves of *Cassia arereh* Del. were carried out. Phytochemical investigation revealed the presence of several bioactive compounds such as alkaloids, flavonoids, anthraquinones, saponins, glycosides, tannins, carbohydrates and terpenes. Elemental analysis revealed the presence of calcium (Ca), phosphorus (P), Manganese (Mg), Manganese (Mn), Iron (Fe), Copper (Cu) and Zinc (Zn). Manganese was present at a higher concentration (11.50 µg/g) followed by Zinc (3.35 µg/g). Antimicrobial studies showed that the ethanolic extract had considerable inhibitory activity against *Staphylococcus aureus*, *Streptococcus pyogen*, *Salmonella typhi*, *Shigella dysentery* and *Eschenchia coli*. Ethanolic extract had higher zone of inhibition (32 mm) against *salmonella typhi* at (0.5 µg/ml) with minimum inhibitory concentration (MIC) value of 8×10^2 µg/ml. The MIC ranges from 4×10^2 µg/ml to 5×10^4 µg/ml for other bacteria.

Keywords: *Cassia arereh*, *Caesalpiniceae*, Medicinal plant, phytochemical, antimicrobial activity.

INTRODUCTION

Man has discovered and used several plants as therapeutic agents from early times. This knowledge of medicinal plants and their related toxic potentials had passed down through generations (Ogarawu, 1992). Of the 300,000 plant species acclaimed world wide, only about 5% have been investigated scientifically for their medicinal purposes (Sanusi and Rabo, 2004). Researchers have also reported that developing countries rely mainly on plants for the treatment of their prevailing ailments especially in areas where hospitals are not accessible (Lambo, 1970). In industrialized countries it is known that over 30% of all prescription drugs came from plant origin (Iwu et al., 1999).

Phytochemical and structural studies on *Cassia italica* (Miller) have resulted in the isolation of 1,5-dihydroxy-3-methylanthraquinone which is a very potent antimicrobial agent (Benjamin, 1980). The phytochemical and cytotoxicity screening of the leaf extracts of *Cassia nigricans* revealed the presence of carbohydrates, anthracene derivatives, cardiac

glycosides, saponins as well as alkaloids (Erickson et al., 1960). From the leaves of *Cassia obovata* 1,8-dihydroxy-3-methylanthraquinone was previously isolated (chrysophanic acid) (Agrawal et al., 2002).

For the past two decades, there has been an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agents (Bonjar and Farrokhi, 2004). *Cassia species* have been of medicinal interest due to their good therapeutic value in folk medicine. Abo et al. (1999) and Elujoba et al. (1999) showed that the leaves and pods of *Cassia fistula*, *Cassia spectabilis*, and *Cassia podacarpa* possess laxative and antimicrobial activities. *Cassia alata* and *Cassia auriculata* were found to possess antidiabetic activity (Jalapure et al., 2004). The pulverized leaves of *Cassia nigricans* are used as appetizers and febrifuges. The leaves and the root powder are used for treating skin diseases such as ringworm, scabies and eczema (Benjamin, 1980).

An infusion is administered as a purgative and vermifuge in Senegal and Chad (Dalziel, 1956, Abegaz et al., 1996). Akah et al. (1998) reported that aqueous extracts of leaves is used by traditional healers in

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Nigeria for the treatment of peptic ulcer. The extract is also used to treat other gastro-intestinal disorders such as stomach aches and diarrhea (Nwafor and Okwuasaba, 2001). Ayo and Amupitan (2007) found out that *Cassia species* contain anthraquinones, flavonoids and polysaccharides and showed considerable antimicrobial activity against Gram-positive microorganisms.

The findings of Abo *et al.* (1999) also show that the extracts from the leaves and pods of *Cassia fistula* and *Cassia spectabilis* showed significant antimicrobial activity. Abo *et al.* (2000) also found out that the methanol extracts of the leaves and pods of *Cassia alata* and *Cassia sieberiana* exhibited significant antimicrobial activity against *P. aeruginosa*, *S. aureus*, *P. mirabilis*, *C. albicans*, *A. niger* and *A. flavus*.

Caesalpiniceae is a family of plant that is found in warm temperate, tropical and subtropical regions of the world. This contains plant species that are potential source of drugs with high antimicrobial activities (Watson and Dallwitz, 1992). *Caesalpiniceae* contain secondary metabolites like alkaloids, tannins, saponins and other phytochemicals which are responsible for their antimicrobial properties (Tsecheshe, 1971).

Cassia arereh Del. (*Caesalpiniceae*) is a woody annual herb or under shrubs between 1.2 and 1.5 m high with small yellow flowers. It is wide spread in India and tropical Africa including northern Nigeria especially in cultivated or old clearings by the road side and open grassy areas (Dalziel, 1956; Irvine, 1961).

The active principles of many drugs found in plants are secondary metabolites. Therefore, basic phytochemical investigation of this extract for major phytochemical constituents is also vital. In the present study, the aqueous and ethanolic extracts of *Cassia arereh* Del. were screened for phytochemical constituents and antimicrobial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Salmonella typhi* and *Shigella dysenteriae*.

MATERIALS AND METHOD

Elemental Determination

Five grams (5 g) of oven dried sample was weighed into a crucible. The crucible was then placed in a hot furnace and ashed at 600 °C for 3 hours. The furnace was cooled to about 120 °C. The crucible was then removed and placed in a desiccator for 1 hour to cool before weighing. The process was repeated until a constant weight was obtained. The ashed samples (0.5 g) was weighed and transferred into the digestion tube. 5 ml each of distilled water, concentrated trioxonitrate (V) acid (HNO₃) and perchloric acid (HClO₄) was added and the content mixed. The tube was placed in the digestion block inside a fume cupboard and the temperature control of the digester was set at 150 °C

and digested for 90 minutes. The temperature was then increased to 230 °C and digested for another 30 minutes (white fuming stage). The digester temperature was reduced back to 150 °C and followed by the addition of 1 ml hydrochloric acid to the tube within a few minutes. The concentrated digest was allowed to cool to room temperature to prevent formation of insoluble precipitate i.e. potassium perchlorate. More water was added to the tube to make up to the mark and the content was mixed and filtered. The resulting solution was used for the elemental analysis using atomic absorption spectrophotometer (AAS) {A. Analyst 400 Model} at an appropriate wavelength, temperature and lamp-current for elements. The following elements were determined – calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn) and phosphorus (P) was determined by UV/V spectrophotometer.

Phytochemical Screening

Plant extracts obtained with ethanol and water were evaluated for the presence of alkaloids, saponins, cardiac glycosides, tannins, steroids and flavonoids (Harborne, J.B. 1973); (Trease, C.E and Evans, W.C 1989)

Saponins: Frothing test – 2 cm³ of the extracts in a test tube was vigorously shaken for two minutes. Frothing observed in each extract tested indicated the presence of saponins.

Emulsion test – 5 drops of olive oil were added to 3ml of the extracts in a test tube and the mixture was vigorously shaken. A stable emulsion formed in each extract tested indicated the presence of saponins.

Tannins: 1 ml of freshly prepared 10% KOH was added to 1 ml of the extracts. A dirty white precipitate observed in each extract showed the presence of tannins.

2 drops of 5 % FeCl₃ were added to 1 ml of the extracts. A greenish precipitate indicated the presence of tannins in each extracts.

Glycosides: 10 ml of 50% H₂SO₄ was added to 10 ml of the extracts in a test tube. The mixture was heated in boiling water for 15 min. 10 ml of Fehling's solution was added and the mixture was boiled. A brick-red precipitate was observed in the methanol and water extracts, showing the presence of glycosides.

Alkaloids: 2 drops of Mayer's reagent were added to 1 ml of the extract. A creamy precipitate observed indicates the presence of alkaloids in each extract.

2 drops of Wagner's reagent were added to 1 ml of each extract. A reddish brown precipitate observed indicates the presence of alkaloids in each extract.

Flavonoids: 1 ml of 10 % NaOH was added to 3 ml of the extracts. A yellow colouration showed the presence of flavonoids in each extract.

Carbohydrates: Few drops of molisch's reagent were added to 2 ml of the extract. 1 ml of concentrated

sulphuric acid was allowed to run down the inclined tube to form a lower layer. The interface was observed for a purple colour showing the presence of carbohydrates.

Anthraquinone: 0.5 g of powdered plant was boiled with 10 ml of ferric chloride (10 %) and 5 ml of dilute HCl for 5 minutes. The mixture was filtered while hot, cooled and the filtrate was shaken with equal volume of chloroform. The layers were allowed to separate in a separating funnel, the chloroform layer was transferred into another test tube containing 5 ml of 10 % ammonia solution and the upper aqueous layer was observed for a bright-pink colour showing the presence of anthraquinone.

Terpenes: To 1 ml of the extract was added 1 ml of acetic anhydride followed by the addition of 1 ml of concentrated sulphuric acid down the wall of the test tube to form a layer underneath. The test tube was observed for red colouration showing the presence of tri-terpenes.

Antimicrobial Screening

Preparation of Agar Medium: 2.5 grams of nutrient agar and 2.6 grams of nutrient broth were added to 100 ml of distilled water in a 500 ml sterilized conical flask. The suspension was heated to dissolve the nutrient agar and broth. After complete dissolution of the media, the mouth of the conical flask was closed tightly with aluminium foil. The media were then sterilized using autoclave at 121 °C, and 15 mmHg for fifteen (15) minutes.

Preparation of Agar Plates: Plates were sterilized in a hot air oven at 160 °C for 2 hours. The plates were allowed to cool. 20 ml of the sterilized nutrient agar was poured into each sterilized plate and the medium was allowed to gel. The agar plates were then wrapped with aluminium foil and transferred into refrigerator until use.

Test Organisms: Cultures of *Staphylococcus aureus*, *Streptococcus pyogen*, *Escherichia coli*, *Salmonella typhi* and *Shigella dysentery* were obtained from University of Maiduguri Teaching Hospital, Maiduguri. All microorganisms were propagated and stored in nutrient agar at 4 °C before use.

Preparation of Stock Solution of Extracts: Stock solutions of extracts were prepared by dissolving 0.2 g of each of the crude extracts in 1ml of the diluents to give a concentration of 200 mg/ml and were kept in sterile coked container until use. Concentration of 300 mg/ml, 400 mg/ml and 500 mg/ml were also prepared together with 250 mg/ml of gentamicin which was aseptically prepared in sterile distilled water and used fresh as the standard antibiotic.

In Vitro Antimicrobial Sensitivity Test: The paper disc diffusion method was used to determine the antimicrobial activity of the test extracts using a standard procedure (Erickson *et al.*, 1960; Bauer *et al.*, 1996). The solution of test extracts of varying

concentrations, ranging from 2×10^5 µg/ml to 5×10^5 µg/ml was prepared. Nutrient agar was prepared, sterilized and used as the growth medium for the microorganisms. The sterilized media (20 ml) were poured into each sterilized petri dish, covered and allowed to gel. The nutrient agar were then inoculated with the test microorganisms and left for about 30 minutes to dry. The sterilized paper discs were soaked in the prepared solutions of the extract with varying concentrations and dried at 50 °C. The dried paper discs were then planted on the nutrient agar seeded with the test microorganism. The plates were incubated at 37 °C for 24 hours, after which they were inspected for the zones of inhibitions using a transparent meter rule. The zones of inhibition were measured and recorded in millimeters (mm).

Minimum Inhibitory Concentration (MIC): This was determined using the broth dilution technique using a standard method (Krivoshan *et al.*, 1989). Solution with a concentration of 200 mg/ml was serially diluted (two fold) to varying concentration ranging from 4×10^2 µg/ml to 1×10^5 µg/ml using nutrient broth and later inoculated with 0.2 ml suspension of the test organism (Usman *et al.*, 2007). The inoculated tubes were then incubated at 37 °C for 24 hours and were inspected for non-turbidity. The least concentration of the extract which prevented visible growth (did not show turbidity) was noted and recorded as the Minimum Inhibitory Concentration (MIC).

RESULTS

Elemental Analysis

The elemental analysis result (Table 1) shows that calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn) and phosphorus (P) were present in all the plant samples at different concentrations.

DISCUSSION

In the present study, elemental analysis, phytochemical screening, antimicrobial activity and minimum inhibitory concentration of aqueous and ethanolic extracts of *Cassia arereh Del.* were carried out. The elemental analysis (Table 1) shows the presence of calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn) and phosphorus (P) in the plant samples. The concentrations of the essential elements appear to be lower which is within safety limit according to WHO (1996). The lower concentration of iron (Fe), zinc (Zn), and copper (Cu) is an indication of little or no toxicity of the plants as heavy metals are known to cause cancer, liver and kidney problems (Ogugbuaja *et al.*, 1997).

The elements (Mg, Ca, Cu, Mn) are used extensively in chemotherapy and are essential in human and animal health. Magnesium and calcium are known to

Table 1. Elemental analysis of the plant samples

Elements	Ca	Mg	Mn	Fe	Cu	Zn	P
Concentrations $\mu\text{g} / \text{g}$	1.056	0.34	11.50	1.54	2.25	3.35	0.247

Table 2. Phytochemical analysis of extract of *Cassia arereh* Del.

Phytochemical constituents	<i>Cassia arereh</i> water extract	<i>Cassia arereh</i> ethanolic extract
Anthraquinone	-	++
Tannins	++	+ ++
Carbohydrates	+	+ + +
Alkaloids	-	++
Glycosides	++	+++
Flavonoids	++	+++
Terpenes	+	+
Saponins	-	++

+++ = High concentration;
 += Low concentration;

++ = Moderate concentration;
 - = Absent

Table 3. Inhibition zone of *Cassia arereh* (Water extract / drug) against the tested microorganisms

Extract /drug (mg/ml)	Zones of inhibition (mm)				
	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogen</i>	<i>E. coli</i>	<i>Shigella dysentery</i>	<i>Salmonella typhi</i>
200	6	9	6	0	16
300	8	15	8	0	18
400	14	18	15	10	24
500	16	21	20	12	26
250 (GTC)	22	32	27	25	35

GTC = Gentamicin

help in bone and teeth development (Khan, 1996; Ogugbuaja *et al.*, 1997).

The phytochemical screening (Table 2) revealed the presence of alkaloids, carbohydrates, tannins, saponins, flavonoids, cardiac glycosides, anthraquinones and tri – terpenes. The ethanol extract of the plant leaves had the most metabolites. This may be as a result of the solubility of the plant extracts in ethanol solvent than the aqueous solvent. Saponins and anthraquinones were not detected in the water extract.

These chemical constituents present in the extracts have many therapeutic values. Tannins and saponins are plant metabolites well known for their antimicrobial properties (Tsechesche, 1971). Flavonoids have both antifungal and antibacterial activity. They possess anti-inflammatory properties (Ogundaini, 2005; Iwu, 1984). It has been reported that cardiac glycosides have specific action on the cardiac muscles and are useful for the treatment of congestive heart failure (Sofowora, 1982). Saponins, flavonoids, terpenes and steroids are known to have antimicrobial and curative properties against several pathogens (Usman *et al.*, 2007; Hassan *et al.*,

2004).

In the anti-microbial studies (Tables 3 - 6) there was a variation in the degree of the antimicrobial activity of the two plant extracts. The variation in the degree could be due to the presence/concentrations of active compounds present in the plant extracts. Majority of the organisms were more sensitive to the ethanol extract of *Cassia arereh*, particularly the gram positive bacteria, this may indicate that the gram positive organisms are more susceptible to the effect of the active compounds in the plants.

The larger zones of inhibition exhibited by the ethanolic extract of *Cassia arereh* may be due to the presence of variety of active compounds in the plants such as tannins, alkaloids, flavonoids and saponins as described by Abo *et al.* (2000). It is not unlikely that one or a combination of the chemical constituents identified through phytochemical screening could be responsible for observed antimicrobial properties of the extracts. Tannins and saponins are plant metabolites well known for their antimicrobial properties (Tsechesche, 1971). Saponins have been used in the treatment of inflammation of the respiratory tract (Trease and Evans,

Table 4. Inhibition zones of *cassia arereh* Del. (ethanolic extract / drug) against tested microorganisms.

Extract/drug (mg/ml)	Zones of inhibition (mm)				
	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogen</i>	<i>E. coli</i>	<i>Shigella dysentery</i>	<i>Salmonella typhi</i>
200	25	18	7	8	27
300	27	20	15	10	30
400	29	23	18	13	30
500	30	23	20	15	32
250 (GTC)	31	25	32	27	35

GTC = Gentamicin

Table 5. Minimum inhibitory concentration (MIC) of *cassia arereh* water extract against the tested microorganisms.

Test organisms	Concentration $\mu\text{g/ml}$				
	3×10^3	6×10^3	1×10^4	3×10^4	5×10^4
<i>Staphylococcus Aureus</i>	-	-	0+	+	+
<i>Streptococcus pyogen</i>	-	-	-	-	0+
<i>E. coli</i>	-	-	0+	+	+
<i>Shigella dysentery</i>	-	0+	+	+	+
<i>Salmonella typhi</i>	-	-	-	-	0+

+ = inhibition; 0+ = minimum inhibition; - = no inhibition (Turbidity)

Table 6. Minimum Inhibitory Concentration (MIC) of *cassia arereh* ethanolic extract.

Test Organism	Concentration $\mu\text{g/ml}$				
	2×10^2	4×10^2	8×10^2	2×10^3	3×10^3
<i>Staphylococcus aureus</i>	0+	+	+	+	+
<i>Streptococcus pyogen</i>	0+	+	+	+	+
<i>E. coli</i>	-	0+	+	+	+
<i>Shigella dysentery</i>	-	0+	+	+	+
<i>Salmonella typhi</i>	-	0+	+	+	+

+ = inhibition; 0+ = minimum inhibition; - = no inhibition (Turbidity)

1989).

From Table 6, it was observed that the ethanol extract of *Cassia arereh* was active against all microorganism. It has MIC of 8×10^2 $\mu\text{g/ml}$ against *Shigella dysentery*, *E. coli*, and *Salmonella typhil*. 4×10^2 $\mu\text{g/ml}$ against *Staphylococcus aureus* and *Streptococcus pyogen*. The standard drug (gentamicin) inhibited the growth of the microorganisms tested in this study.

These findings were consistent with those of Singh and Agrawal (2000), who observed that *Cassia species* containing anthraquinones, flavonoids and reducing sugar showed considerable antimicrobial activity against gram positive microorganisms.

Abo (1999) also showed that the extract from the leaves and pods of *Cassia fistula*, *Cassia podocarpa*

and *Cassia spectabilis* showed significant antimicrobial activity. Abo *et al.* (2000) also found that the ethanol extract of the leaves of *Cassia sieberiana* exhibited significant antimicrobial activity against *P. aeruginosa*, *S. aureas* and *P. mirabiis*.

CONCLUSION

The results of the present experiment showed that the leaf of *Cassia arereh*, have anti-microbial potentiality against gram positive and gram negative microorganisms. This property tends to support the traditional medicinal use of the plant in the treatment of bacterial infections. Finally, it is apparent from our study that effective drugs could be produced from *Ceasalpin-*

iaeceae family of plants used in traditional medicine. This could lead to development of local pharmaceutical industries, thereby enhancing self reliance and reduce drug importation.

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