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Antimicrobial activity and Elemental analysis of *Cassia siberiana* leaves Using Atomic Absorption Spectrometer

²Olajide Olutayo, ²Afolayan Michael, ³Adewusi Adepoju John, ¹Adeyanju Olusola*

¹Department of Chemistry, University of Jos, Nigeria

²Chemistry Advanced Laboratory, Sheda Science and Technology Complex, Sheda, Abuja, Nigeria

³Department of Pure and Applied Chemistry, Ladoké Akintola University of Technology, Ogbomosho, Nigeria

ABSTRACT

Phytochemical screening and elemental analysis was carried out on water and ethanol extracts of *Cassia siberiana* leaves. The antimicrobial activity and minimum inhibitory concentrations (MIC) of the leaves extract were also studied. Preliminary phytochemical investigation revealed the presence of alkaloids, flavonoids, anthraquinones, saponins, glycosides, tannins, carbohydrates and terpenes. Elemental analysis revealed the presence of calcium (Ca), phosphorus (P), Magnesium (Mg), Manganese (Mn), Iron (Fe), Copper (Cu) and Zinc (Zn). Manganese was present at the higher concentration of 9.50 µg/g followed by Zinc 2.4 µg/g. Antimicrobial studies showed that the ethanol extract had considerable activities and significant inhibition against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhi*, *Shigella dysenteriae* and *Escherichia coli*. Ethanol extract had higher zone of inhibition (32mm) against *Salmonella typhi* at (0.5 µg/ml) with minimum inhibitory concentration (MIC) value of 4×10^2 µg/ml. The MIC ranges from 2×10^2 µg/ml to 5×10^4 µg/ml.

Key words: *Cassia siberiana*, *Caesalpiniceae*, Medicinal plant, phytochemical, antimicrobial activity.

INTRODUCTION

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 reported that developing countries rely mainly on plant 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 are from plant origin (Iwu *et al.*, 1999).

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 podocarpa* 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). Akahet *et al.* (1998) reported that the aqueous extracts of the leaves is used by traditional healers in Nigeria for the treatment of peptic ulcer. The extract is also used to treat other gastro-intestinal disorders such as stomach aches and diarrhoea (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.

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 contains secondary metabolite like alkaloids, tannins, saponins and others which are responsible for their antimicrobial properties (Tsecheshe, 1971).

Cassia siberiana (Caesalpiniceae) is a woody annual herb or under shrubs between 1.4 m and 1.7 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 plant are secondary metabolites. Therefore, basic phytochemical investigation of this extract for major phytochemical constituents is also vital in the present study, the water and ethanolic extracts of *Cassia siberiana* were screened for phytochemical constituents and antimicrobial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Salmonella typhi* and *Shigella dysentery*.

MATERIALS AND METHODS

Elemental Determination

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

control of the digester was set at 150 °C and digested for 90 mins. The temperature was then increased to 230 °C and digested for another 30 mins (white fuming stage). The digester temperature was reduced back to 150 °C, and followed by the addition of 1 ml of hydrochloric acid to the tubes within a few mins. The concentrated digest was not 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 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 the different elements. The following elements were determined, calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn) but 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 mins. Frothing observed in the three extracts 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 the three extracts.

Glycosides:

10ml of 50% H₂SO₄ was added to 10 ml of the extracts in a test tube. The mixture was heated in boiling water for 15 mins. 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 1ml of the extract. A creamy precipitate observed indicates the presence of alkaloids in each extract.

2 drops of Hager's reagent were added to 1ml 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 extract. A yellow colouration showed the presence of flavonoids in each extract.

Carbohydrate:

Few drops of molisch's reagent was 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 carbohydrate.

Anthraquinone

0.5 g of powdered plant was boiled with 10 ml of ferric chloride (10%) and 5 ml of dilute HCl for 5 mins. 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 anthraquinones.

Terpenes

To 1 ml of the extract was added 1 ml acetic anhydride followed by the addition of 1 ml 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 g of nutrient agar and 2.6 g 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 was then sterilized using autoclave at 121 °C, and 15 mmHg for fifteen (15) mins.

Preparation of Agar Plates

Plates were sterilized in a hot air oven at 160 °C for 2 h. 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 a refrigerator until use.

Test Organisms

Cultures of *staphylococcus aureus* , *streptococcus*, *Eschericia coli*, *salmonella* 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 1 ml of the diluents to give a concentration of 200 mg/ml and were kept in sterile cocked container until use. Concentrations 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 solutions of test extracts of varying concentrations, ranging from 2×10^5 $\mu\text{g/ml}$ to 5×10^5 $\mu\text{g/ml}$ were 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 allow to gel. The nutrient agar were then inoculated with the test microorganisms and left for about 30 mins to dry. The sterilized paper discs were soaked in the prepared solutions of the extract with varying concentration 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 h, 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 $\mu\text{g/ml}$ to 1×10^5 $\mu\text{g/ml}$ using nutrient broth and later inoculated with 0.2ml 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 in (Table I) shows that calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn) and phosphorus (P) were present in all the plant samples at different concentrations.

Table I: Elemental analysis of the leaf samples

Sample(s)	Ca	Mg	Mn	Fe	Cu	Zn	P
Concentration ($\mu\text{g} / \text{g}$)	1.056	0.34	9.50	1.54	2.25	2.40	0.247

Table II: phytochemical analysis of extract of *Cassia siberiana*

Phytochemical constituents	(water extract)	(ethanolic extract)
Anthraquinone	-	++
Tannins	++	+ ++
Carbohydrate	+	+ ++
Alkaloid	-	++
Glycoside	++	+++
Flavonoids	++	+++
Terpenes	+	+
Saponins	-	++

+++ = High concentration; ++ = Moderate concentration + = Low concentration; - = Absent

Table III: Inhibition zone of *Cassia siberiana* (Water extract / drug) against the tested microorganism

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

Table IV: Inhibition zones of *Cassia siberiana* (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	14	35
250 (GTC)	31	25	30	25	35

GTC = Gentamicin

Table V: Minimum inhibitory concentration (MIC) of *cassia siberiana* 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 VI: 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 dysentary</i>	-	0+	+	+	+
<i>Salmonella typhi</i>	-	0+	+	+	+

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

DISCUSSION

In this research, elemental analysis, phytochemical screening, antimicrobial activity and minimum inhibitory concentration of water and ethanol extracts of *Cassia siberiana* were carried out. The elemental analysis result (Table I) shows the presence of calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn) and phosphorus (P) at different concentrations in the plant

samples. All the elements tested for were present in *Cassia siberiana*. The concentrations of the essential elements appear to be lower which is within safety limit according to W.H.O (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 help in bone and teeth development (Khan, 1996; Ogugbuaja *et al.*, 1997).

The phytochemical screening (Table II) revealed the presence of alkaloids, carbohydrates, tannins, saponins, flavonoids, cardiac glycosides, anthraquinones and triterpenes. 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. Saponin and anthraquinone 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 property (Ogundaini, 2005; Iwu, 1984). It was reported that cardiac glycosides have specific action on the cardiac muscles and they 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 (Table III - VI) 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 different active compounds present in the plants. Majority of the organisms were more sensitive to the ethanol extract of *Cassia siberiana*, 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 siberiana* 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. This is more so since 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, 1989).

From the results obtained, the leaf extract of *Cassia siberiana* showed more antimicrobial activity even at low concentration. This suggests that *Cassia* leaf contains more of the active compounds and has high potency.

In Table VI, the ethanol extract of *Cassia siberiana* was active against the entire microorganism. *Staphylococcus aureus*, *Streptococcus pyogenes*, *E. coli*, *Shigella dysentery* and *Salmonella thypi*. It has MIC of 4×10^2 $\mu\text{g/ml}$ against *Shigella dysentery*, *E. coli*, and *Salmonella thypi*, 2×10^2 $\mu\text{g/ml}$ against *Staphylococcus aureus* and *Streptococcus pyogenes*. The standard drug (gentamicin) inhibited the growth of the microorganisms tested in this study.

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

They also agreed with the findings of (Abo *et al.*,1999) 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 singueana Del* exhibited significant antimicrobial activity against *P. aeruginosa*, *S. aureas* and *proteus mirabiis*.

CONCLUSION

The results of the experiment showed that the leaf of *Cassia siberiana* may have some value anti-microbial activities against gram positive and gram negative microorganisms. This property tends to support the traditional medicinal stage in the treatment of bacterial infections. The result of the study justified the use of the plant extracts in the treatment of diseases of microbial origin in herbal medicine.

Finally, it is apparent from our study that effective drugs could be produced from *Ceasalpiniaeceae* family of plants used in traditional medicine. This could lead to development of local pharmaceutical industries, thereby enhanced self reliance and reduced drug importation.

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