Research Article

Antimicrobial Activity of *Cassia occidentalis* Seed Extract.

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ABSTRACT

Hexane and ethanol extracts from the seed of *Cassia occidentalis* were evaluated for their antimicrobial activity *in vitro* using agar diffusion method. The seed of *Cassia occidentalis* at concentration ranging from 50-200 mg/ml were tested against some pathogenic organisms such as *Escherichia coli,* Pseudomoneas *aerugimosa, Candida albican, Salmonella typhi and Staphycoccus aureus*. Both extracts exhibited antibacterial activity against the tested organisms. It showed highest antibacterial activity against *Pseudomonas aeruginosa* and *Escherichia* coli. The extracts exhibited antibacterial activities with the zones of inhibition ranging from 8-20 and 6-15 mg/ml against *Pseudomonas aeruginosa* and *Escherichia coli re*spectively. The minimum inhibitory concentration (MIC) was 6.25 mg/ml and 12.5 mg/ml for *P. aeroginosa* and *E.coli* respectively which was determined using the broth dilution method; the minimum bactericidal concentration (MBC) ranges from 50- 25 mg/ml. Phytochemical screening of the crude extract revealed the presence of carbohydrate, terpene, steroid, sugar and tannins. The result obtained from this study suggests that the plant could be a potential source of drug for the treatments of diseases associated with the test organisms.

INTRODUCTION

Cassia occidentalis also known as Fedegoso is a leguminous plant belonging to the family leguminoseae. Many species have been used medicinally, various cassia plants have been used as purgatives and laxatives, including *Cassia angustifolia* and *Cassia senna*. In Cuba, *C. occidentalis* is said to be most common[1]. Its roots, leaves, flowers, and seeds have been employed in herbal medicine around the world. In peru, the roots are considered a diuretic, and a decoctoin is made for fevers. The seeds are brewed into a coffee-like beverage for asthma and a flower infusion is used or bronchitis. In Brazil, the roots of Fedegoso are considered a tonic, fever reducer, and diuretic; they are used for fevers, menstrual problems, tuberculosis, anemia, liver complaints, and as a tonic for general weakness and illness. The leaves are also used in Brazil for gonorrhea, fevers, urinary trait disorders, oedema, and menstral problems. The miskito Indians of Nicaragua use a fresh plant decoction for general pain, menstrual and uterine pain, and constipatoin in babies. In Panama, a leaf tea is used for stomach colic, the crushed leaves are used in a poultice as an anti-inflammatory and the crushed fresh leaves are taken internally to expel intestinal worms and parasites (http://www.raintree.com/fedegosa.htm. 7p). In many countries around the world, the fresh and/or dried leaves of Fedegoso are crushed or brewed into a tea and applied externally for skin fungus, parasitic skin diseases, abscesses and as a tropical analgesic and anti-inflammatory natural medicine (http://www.rain-tree.com/fedegosa.htm.7p).

Fedegoso was able to prevent or reduce the mutation of healthy cells in the presence of laboratory chemicals which were known to mutate them. Fedegoso leaf extracts have demonstrated an anti-inflammatory, hypotensive smooth-muscle relexant, antisepasmodic, weak uterine stimulant, vasoconstrictor, and antioxidant activities in laboratory animals (http://www.rain-tree.com/fedegosa.htm.7p). Toxicity studies on the aerial parts, leaves, and roots of fedegoso have been published by several research groups. These studies reported that various leaf and root extracts given to mice (administered orally and injected at up to 500 mg/kg) did not demonstrate any toxic effect or cause mortality (http://www.rain-tree.com/fedegosa.htm.7p). In the present work an attempt was made to study the antimicrobial activity of seed extracts of *Cassia occidentalis*.

MATERIALS AND METHODS

Preparation of Extract

The soxhlet extraction technique was used. Exactly 30g of the powdered seed sample was extracted using 300ml of 90% hexane and 10% acetone. The extract was placed on a water bath for all the solvent to evaporate and oil retained. The oil produced was stored in a refrigerator at 4^0C in dark tightly stoppered glass until analysis.

The defatted extract was air-dried at room temperature,weighed and further extraction was done using soxhlet technique and ethanol (300 ml) as a solvent. This was placed in an air tight stoppered glass ware before analysis.

Phytochemical screening

Phytochemical screening of the extracts was carried out according to the methods described by Sofowora [2] and Trease and Evans[3] for the detection of active components like saponins, tannins, alkaloids, phlobatannins, glycosides and etc. To detect the presence of tannins- 1 cm^3 of freshly prepared 10 % KOH was added to 1 ml of the extract. A dirty white precipitate showed the presence of

tannins. The presence of Glycosides was observed by the appearance of a brick-red precipitate. For saponin, Frothing test: 2 ml of the extract was vigorously shaken in the test tube for 2 minutes. Presence of frothing indicates saponins. Yellow colouration is indicative of the presence of flavonoids. For Steroids, 5 drops of concentrated H_2SO_4 was added to 1 cm^3 of the extract in a test tube. Red colouration indicate the presence of steroids. Blue-green colour indicates the presence of triterpenes. For phenolics, two drops of 5 % $Fecl_3$ was added to 1 cm^3 of the extract in a test tube. Presence of greenish precipitate indicates the presence of phenolics. The presence of carbohydrate was observed by boiling 3 g of the powdered sample in 50 ml of distilled water on a hot plate for three minutes. The mixture was filtered while hot and the resulting filtrate was cooled. A few drops of Molisch's reagent was added to 2 ml of the warm extract, then a small quantity of concentrated sulphuric acid was added to form a lower layer. A purple ring at the interface indicates the presence of carbohydrates.

Preparation of Culture

Organism collected include: *Pseudomonas aeruginosa, Escherichia coli, Candida albican, Staphylococcus aureus* and *Salmonella typhi*

The stock culture of bacteria and fungi were collected from Microbiology Dept. of National Institute for Pharmaceutical Research Development (NIPRD) Abuja and maintained on Nutrient Agar and Potato Dextrose Agar at 28^o C for 48 hrs respectively. The colonies of each pure culture were inoculated into 5 ml sterile broth in a McCartney bottle and it was incubated over night at $37\ ^oc$.

Standardization of inoculums

5.0 ml of sterile nutrient broth was inoculated with a loopful of test organism and incubated for 24 hrs. 0.2 ml from the overnight culture of the organisms were dispensed into 19.0 ml of sterile nutrient broth and incubated for 3-5 hrs by using Mcfarland standard to standardise the culture to 10^6 cfu/ml a loopful of the standard culture was used for the antimicrobial assay.

Antimicrobial assay

Mueller Hinton agar was prepared according to the manufacture's direction (19 ml each was dispense into McCartney bottles), 1 ml of standardised test organism was inoculated into the molten agar and was poured into sterile Petri dishes which was allowed to gel at room temperature, sterile cork borer was used to make wells which base was covered with molten agar, different concentration of the prepared extracts was introduce into the wells and it was allowed to pre diffuse for 30 mins. The bacteria and fungi plates were then incubated at $37\ ^oc$ for 24 hours and 48 hours respectively after which the zone of inhibition was measured and recorded in millimetre (mm).

Determination of the Minimum Inhibitory Concentration (MIC) of the Crude Extract.

The broth dilution method was employed, 9 ml of nutrient broth was dispensed into several number of test tubes, this was sterilized at $121\ ^oc$ for 15 mins ,this was allowed to cool to room temperature and was labelled 1,2 3….10. 1ml from the re-constituted extracts was introduced into test tube 1 and the rest tubes. 1ml of the standardized inoculums was introduced into the broth, the tubes were incubated for 24 hrs and turbidity was observed. The tube with no visible growth was taken and recorded as the MIC.

Determination of MBC

The tube with no visible growth was sub cultured into a freshly prepared nutrient agar and incubated. The plate with the

least or no growth was recorded as the MBC

RESULTS

A result of Phytochemical screening of *Cassia occidentalis* seed extracts is presented in (**Table 1**). The percentage yield for hexane and ethanol extracts was 1.4 and 1.3 respectively. The major components present in hexane extract were terpenes, steroids carbohydrates and sugars, the least was tannins. Alkaloids, saponins and glycosides were all absent in hexane extract. Ethanol extract contained tannins and sugars as the major components. terpenes, steroids carbohydrates were sparsely present. Alkaloids, saponins and glycosides were similarly absent in ethanol extract (**Table 1**). Antimicrobial activity of ethanol extract of *C .occidentalis* is presented in (**Table 2**). The activity of the extract on the tested microorganisms increases with increase concentrations. The highest activity was observed in *Pseudomonas aeruginosa* (20 mm, 15 mm) followed by *E. coli* (15 mm, 11 mm) at 200 mg/ml and 500 µl respectively. The results were comparable with the control (**Table 2 and 3**). There was no activity on *candida albican, Salmonella typhi and Staphycoccus aureus*. The results of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) are presented in (**Table 4)**. The MIC was 6.25 mg/ml and 12.5 mg/ml for *P. aeroginosa* and *E.coli* respectively. MBC ranges from 50- 25 mg/ml for both *P. aeroginosa* and *E. coli*.

Table 1 Results of Phytochemical screening of *Cassia occidentalis* seed extracts

Extracts	% yield	Secondary metabolites							
		Alkaloid	**Saponin**	**Terpene**	**Steroid**	**Tannins**	**CHO**	**Glycoside**	**Sugar**
COH	1.4	—	—	++	++	+	++	—	++
COE	1.3	—	—	+	+	++	+	—	++

COH (*Cassia occidentalis* hexane extract), COE (*Cassia occidentalis* Ethanol extract), CHO (Carbobydrate), + Slightly present, ++ Highly present, -Absent

Table 2 Antimicrobial activity of ethanol extract of *C.occidentalis* (zone of inhibition in mm)

Isolates	Ethanol extracts concentrations			
	200 mg/ml	**100 mg/ml**	**50 mg/ml**	**+control, (25 mg/ml)**
P. aeruginosa	20	16	8	10
E.coli	15	10	6	20
C. albican	_	_	_	_
S. aureus	_	_	_	24
S. typhi	_	_	_	17

No activity, +ve control: ciprofloxacin, NT: Not Tested

Table 3 Antimicrobial activity of hexane extracts (oil)

Isolates	Hexane Extracts Concentrations.			
	(500 µl)	(250 µl)	(100 µl)	control
P. aeruginosa	15	10	3	NT
E.coli	11	7	0	NT
C. albican	0	0	0	20
S. aureus	0	0	0	NT
S. typhi	_	_	_	NT

No activity, +ve control: ciprofloxacin, NT: Not Tested

Table 4 Minimum inhibitory concentration (MIC) and MBC

Conc.	*E. coli*		*P. aeruginosa*	
	MIC	*MBC*	MIC	MBC
50	_	_	_	_
25	_	+	_	_
12.5	_	+	_	+
6.25	+	++	_	+
3.13	++	++	+	++
1.56	++	++	+	++
0.78	++	++	++	++

- not turbid, + turbid, ++ very turbid

DISCUSSION

The phytochemical screening in (**Table 1**) showed the presence of carbohydrate, reducing sugar, terpene and steroid as the active component of the plant. A lot of research has been carried out on the screening of different medicinal plants for active phytochemicals [4, 5]. These phytochemicals are thought to be responsible for the medicinal effects of these plants [8]. Several phenolic compounds like tannins present in the cells of plants are potential inhibitors of many hydrolytic enzymes such as pectolytic macerating enzymes used by plant pathogens. It was also showed that component like saponin, glycosides, alkaloid and anthraquinone were absent. Many plants contain non-phlobatanin which can get hydrolyse to release phenolics which are toxic to microbial pathogens [6]. Some of these compounds are likely to be active against certain bacteria, this may account for the traditional used as medicinal plants. Traditional medicine practitioner often uses two or more plants in synergy to treat infections caused by a variety of bacteria [6].

As showed in (**Table 2, 3 and 4**) the results of the various tests carried out in this investigation clearly demonstrated the antimicrobial potential of the hexane and ethanol extracts on *E. coli,* and *P. aeruginosa*. It indicates that only two out of the five organisms tested were sensitive to the extracts at the tested concentrations.

CONCLUSION

The inhibitory effect of the extracts of *C. occidentalis* against *E. coli* and *P. aeruginosa* can introduce the plant as a potential candidate for drug development for the treatment of ailments caused by these pathogens. This seed has also shown to have active secondary metabolites which may be responsible for its activity. This result authenticates its use in traditional medicine.

It is known that herbal medicine has good values, in treating many diseases but there are many challenges to practice of herbal medicine. Despite the challenges, herbal medicine afford clinical and research opportunities that should not be neglected when greater regulation of product is considered. Without doubt, the therapeutic potential of many herbs is yet to be fully discovered

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