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The biocidal and phytochemical properties of leaf extract of *Cassia occidentalis* linn.

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The antibacterial potentials of *Cassia occidentalis* leaf extracts were investigated against eleven Gram-positive and four Gram-negative bacterial isolates. The n-hexane and dichloromethane fractions of the plant extract exhibited appreciable antibacterial action against nine out of the fifteen bacterial isolates tested at a concentration of 20 mg/ml. The zones of inhibition exhibited by n-hexane fraction ranged between 16 and 27 mm while that of dichloromethane fraction ranged between 15 and 28 mm. On the other hand, the zones of inhibition observed for the standard antibiotic, streptomycin ranged between 13 and 30 mm. The minimum inhibitory concentrations exhibited by n-hexane and dichloromethane fractions against the susceptible test isolates ranged between 0.157 and 1.25 mg/ml, respectively. Overall, the two fractions compared favourably with the standard antibiotic, streptomycin used in this study. The phytochemical analysis of the extract revealed the presence of tannins, Saponins, anthraquinones and flavonoids.

Key words: *Cassia occidentalis*, antibacterial activity, phytochemical compounds, National Collection for Industrial Bacteria (NCIB), locally isolated organism (LIO).

INTRODUCTION

Multiple resistance to currently available antibiotics by pathogens responsible for various diseases in man is increasing at an alarming rate and therefore the need to source for antimicrobial agents of natural origin especially from enormous medicinal plants around us. This study is therefore one of the approaches to solve such problems. *Cassia occidentalis* is a shrub that grows between 5 to 8 cm in height and belongs to the family Caesalpiniaceae and commonly found in the tropics (Kaey, 1989). It is in the same genus as Senna and sometimes called coffee senna. The leaves of the plant are used for the treatment of yaws, scabies, itches and ringworm among the Yoruba tribe of southwestern Nigeria. In addition to this, the leaves are also known to be effective against jaundice, headache and toothache. Infusion of *C. occidentalis* leaves is used as an effective treatment for hepatitis among the rural dwellers in northern part of Nigeria (Nuhu and Aliyu, 2008). *C. occidentalis* leaves have ethno medical importance like wound healing, treatment of sores, itch, cutaneous diseases, bone fracture, fever, ringworm and throat infection (Jain et al., 1998; Burkill, 1995). *C. occidentalis* is used as a diuretic and in the treatment of snake-bite (Yadava and Satnami, 2011). Different parts of this plant have been reported to possess anti-inflammatory and antiplasmodial activities (Kuo et al., 1996; Tona et al., 2004). From the study carried out by Verma et al. (2010), they observed that ethanolic extract of this plant exhibited significant

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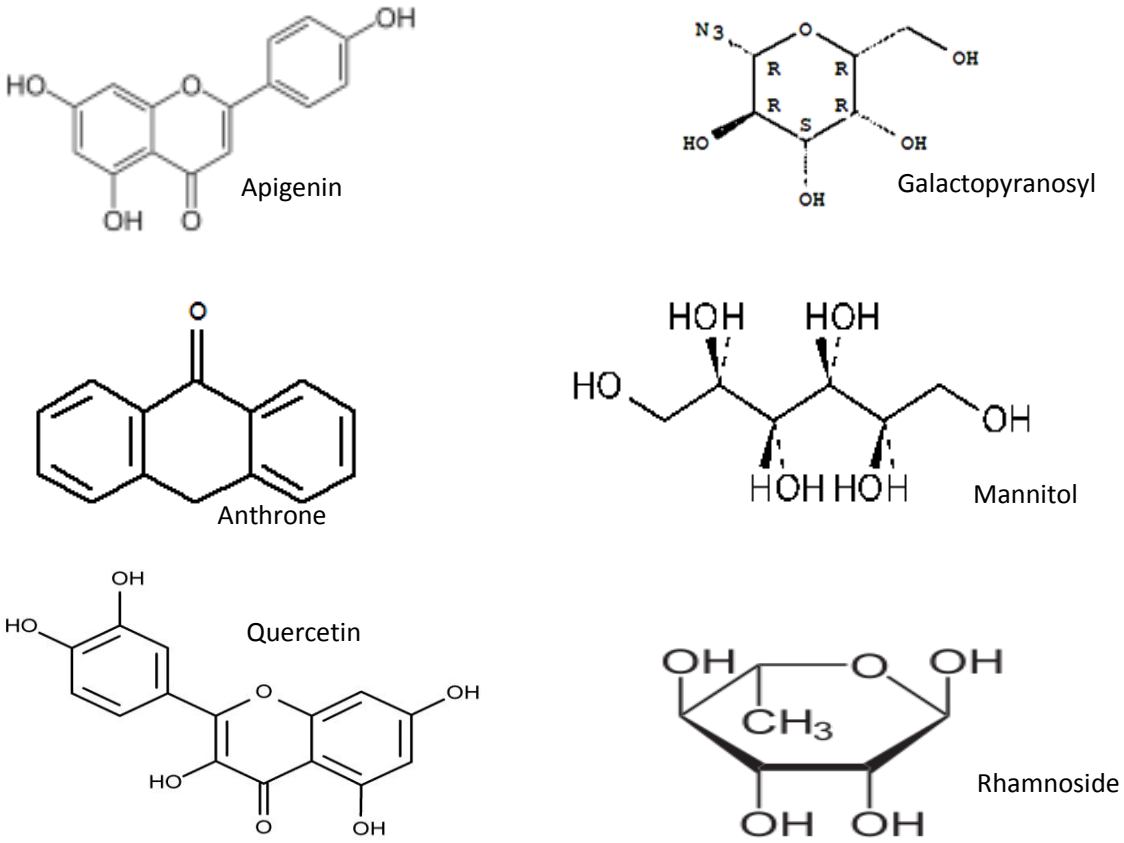

Figure 1. Structures of chemical compounds isolated from *Cassia occidentalis* extracts. Source of chemical structures: www.chemicalbook.com.

antidiabetic activity in normal and alloxan-induced diabetic rats. Bin-Hafeez and Hussaini (2001) have also shown that extract obtained from *C. occidentalis* was useful as a cellular protector and preventive to cell damage. Sadiq et al. (2012) reported the susceptibility of *Sallmonella typhi* and *Shigella* species to the ethanolic extract of *C. occidentalis*. The phytochemical compounds present in this extract include tannins, saponins, cardiac glycoside, terpenoids and anthraquinones. Structures of some of the chemical compounds isolated from different parts of *C. occidentalis* are shown in Figure 1.

The objective of this work is to test for the antimicrobial activities of different fractions of *C. occidentalis* against some bacterial isolates and to screen for the phytochemical compounds in this plant.

MATERIALS AND METHODS

Plant sample

C. occidentalis leaves were collected from Agriculture Research Farm, Obafemi Awolowo University, Ile Ife, Nigeria in June, 2012. The plant was identified in Department of Botany Herbarium, Obafemi Awolowo University, Ile Ife, Nigeria by Dr H. I. Illoh. The sample was oven-dry at 40°C until the constant weight was observed. The dried leaves was powdered and stored in an air-tight container for further use.

Preparation of extract

About 720 g of the powdered leaves of *C. occidentalis* was extracted in cold using 60% methanol for 4 days. The mixture was then filtered and the filtrate was dried *in vacuo* using a rotary evaporator. The yield collected was 87 g.

Preparation of bacterial isolates for experiment

The following organisms were used for the experiment.

Gram-positive organisms

Bacillus subtilis (NCIB 3610), *Bacillus cereus* (NCIB 349), *Bacillus stearothermophilus* (NCIB 8222), *Micrococcus luteus* (NCIB 196), *Bacillus anthracis* (LIO), *Staphylococcus aureus* (NCIB 8588), *Enterococcus faecalis* (NCIB 775), *E. faecalis* (LIO), *Clostridium sporogenes* (NCIB 532), *Corynebacteriun pyogenes* (LIO) and *B. polymyxa* (LIO).

Gram-negative bacteria

Pseudomonas aeruginosa (NCIB 950), *Klebsiella pneumonia* (NCIB 418), *Escherichia coli* (NCIB 86) and *Pseudomonas flourescens* (NCIB 8756).

Sensitivity testing of fractions obtained from *C. occidentalis* leaf extract on test isolates

The antimicrobial activities of the fractions were determined using agar-well diffusion method described by Russell and Furr (1977) and Irobi et al. (1994) with little modification. The bacterial isolates were first grown in nutrient broth before use and later standardized using Mac Farland test. One hundred microliter (100 µL) of the standardized bacterial suspension was evenly spread on Mueller-Hinton agar medium using a sterile glass spreader. Wells were then bored into the agar medium using a sterile 6 mm cork borer and were filled with the solution of the fractions taken care not to allow spillage of the solution to the surface of the agar medium. The plates were allowed to stand on the laboratory bench for 1 h to allow for proper diffusion of the extract into the medium. The plates were later incubated in an incubator at 37°C for 24 h after which they were examined for zones of inhibition. The effects of the fractions on the test isolates were compared with that of the standard antibiotic, streptomycin.

Determination of the minimum inhibitory concentrations (MICs)

The MICs of the fractions were determined using two-fold dilutions of the fractions as described by Akinpelu and Kolawole (2004). Two-fold of the fraction was prepared and 2 ml of different concentrations of the solution was added to 18 ml of pre-sterilized molten nutrient agar at 40°C to give final concentrations regime of 10 to 0.079 mg/ml. The medium was then poured into Petri dishes and allowed to set. The surfaces of the media were allowed to dry before streaking with 18 h old bacterial isolates. The plates were later incubated in an incubator at 37°C for up to 72 h after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that will prevent the growth of the test bacterial isolates.

Determination of minimum bactericidal concentrations (MBCs)

The MBC of the extract was determined using Olorundare et al. (1992) with little modification. Samples were taken from plates with no visible growth in the MIC assay and sub-cultured onto freshly prepared nutrient agar plates and later incubated at 37°C for 48 h. The MBC was taken as the concentration of the extract that did not show any growth on a new set of agar plates.

Preliminary phytochemical analysis of *C. occidentalis* extracts

A small portion of the dry extract was subjected to the phytochemical test using Trease and Evans (1983) and Harborne (1998) methods to test for alkaloids, tannins, flavonoids, steroids, saponins, reducing sugars and cardiac glycoside.

Test for alkaloids

Exactly 0.5 g of the plant extract was dissolved in 5 ml of 1% HCl on steam bath. A millilitre of the filtrate was treated with drops of Dragendorff's reagent. Turbidity or precipitation was taken as indicative of the presence of alkaloids.

Test for tannins

About 1 g of the extract was dissolved in 20 ml of distilled water and filtered, 2 to 3 drops of 10% of $FeCl_3$ was added to 2 ml of the filtrate. The production of a blackish-blue or blackish-green colouration was indicative of tannins. To another, 2 ml of the filtrate was added 1 ml of bromine water. A precipitate was taken as positive for tannins.

Test for flavonoids

A 0.2 g of the extract was dissolved in 2 ml of methanol and heated. A chip of magnesium metal was added to the mixture followed by the addition of a few drops of concentrated HCl. The occurrence of a red or orange colouration was indicative of the flavonoids.

Test for saponins

Freshly prepared 7% blood agar medium was used and wells were made in it. The extract in methanol was applied with distilled water and methanol used as negative control while commercial saponin (BDH) solution was used as positive control. The plates were incubated at 35°C for 6 h. Complete haemolysis of the blood around the extract was indicative of saponins.

Test for anthraquinones

About 1.0 g of the plant extract was dissolved in petroleum ether and filtered. Aqueous ammonia was then added to the filtrate, formation of pink colouration was taken as indication for the presence of anthraquinones in the plant extract.

RESULTS AND DISCUSSION

The results obtained from our investigations on two fractions (n-hexane and dichloromethane) of *C. occidentalis* extract revealed that both fractions exhibited a broad spectrum activity against the test bacterial isolates (Tables 2 and 3). The two fractions exhibited appreciable antimicrobial activities at a final concentration of 20 mg/ml. The zones of inhibition exhibited by dichloromethane fraction of the extract against Gram-positive bacteria ranged between 15 and 24 mm, while it was between 20 and 24 mm for Gram-negative bacteria. On the other hand, zones of inhibition exhibited by n-hexane fraction of the extract against Gram-positive bacteria ranged between 16 and 27 mm, while the zones are between 20 and 25 mm against the Gram-negative bacteria. The standard antibiotic streptomycin, used as positive control exhibited zones of inhibition ranging between 15 to 27 and 14 to 30 mm against the Gram-positive and Gram-negative bacterial isolates, respectively. From the results obtained, the two fractions compared favourably with the standard antibiotic used in this study. Among the tested isolates susceptible to the antimicrobial effects of the two fractions extracted from *C. occidentalis* is *P. aeruginosa*, known to be resistant to many antibiotics (Pelczar et al., 2006) and this was observed against streptomycin in this study. *E. coli*, *K. pneumoniae* and *M. luteus*, which are all known to cause ailments in man were also susceptible to these fractions. Thus, the study supports the usefulness of *C. occidentalis* in folklore remedies for the treatment of disease caused by these pathogens.

The MICs of both dichloromethane and n-hexane fractions were also determined. The MIC exhibited by dichloromethane and n-hexane fractions against the susceptible bacterial isolates ranged between 0.157 and 1.25

Table 1. Phytochemical compounds present in the leaf extract of *C. occidentalis*.

Phytochemical compound	Test	Observation	Indication
Tannins	Ferric Chloride	Blue-green colour	Positive
Anthraquinones	Borntrager	Bright pink colour	Positive
Saponins	Frothing test	Frothing formation	Positive
Flavonoids	HCl	Red or Orange colour	Positive
Alkaloids	Meyer	Turbidity	Negative

Table 2. Sensitivity pattern of zones of inhibition exhibited by *C. occidentalis* leaf fractions and standard antibiotics against the Gram-positive bacterial isolates.

Microorganism	Zones of inhibition (mm)*		
Bacterial isolate	**DCM fraction (20 mg/ml)**	**NHX fraction (20 mg/ml)**	**STP (10 µg/ml)**
Bacillus subtilis (NCIB 3610)	0	0	27
Bacillus cereus (NCIB 349)	0	0	24
Bacillus stearothermophilus (NCIB 8222)	23	25	23
Micrococcus luteus (NCIB 196)	18	21	13
Bacillus anthrax (LIO)	15	18	18
Staphylococcus aureus (NCIB 8588)	0	0	24
Enterococcus faecalis (NCIB 775)	0	0	23
Enterococcus faecalis (LIO)	20	16	21
Clostridium sporogenes (NCIB 532)	15	18	25
Corynebacterium pyogenes (LIO)	0	0	20
Bacillus polymyxa (LIO)	24	27	15

Key: DCM = dichloromethane;NHX = n-hexane; STP = streptomycin; LIO = locally isolated organisms; NCIB = National Collection of Industrial Bacteria; *****mm **=** mean of three replicates.

Table 3. Sensitivity pattern of zones of inhibition exhibited by *C. occidentalis* leaf fractions and standard antibiotics against the Gram-negative bacterial isolates.

Microorganism	Zones of inhibition (mm)*		
Bacterial isolate	**DCM fraction (20 mg/ml)**	**NHX fraction (20 mg/ml)**	**STP (10 µg/ml)**
Pseudomonas aeruginosa (NCIB 950)	20	23	ND
Klebsiella pneumoniae (NCIB 418)	22	20	14
Escherichia coli (NCIB 86)	28	25	18
Pseudomonas flourescens (NCIB 8756)	0	0	30

Key: DCM = dichloromethane; NHX = n-hexane; STP = streptomycin; LIO = locally isolated organisms; NCIB = National Collection of Industrial Bacteria; ND = Not done; *****mm **=** mean of three replicates.

mg/ml (Table 4). This is an indication that the two fractions exhibited almost the same antibacterial effects against the susceptible isolates. Minimum bactericidal concentrations (MBCs) of the two fractions were also determined. The MBC exhibited by dichloromethane fraction against the susceptible organisms ranged between 0.313 and 2.50 mg/ml. On the other hand, the MBC exhibited by n-hexane fraction against the isolates was between 0.313 and 5.0 mg/ml (Table 5). This is a reflection of what was observed for the MICs results. Investigation on the phytochemical compounds of *C. occidentalis* leaf extract revealed the presence of tannins, anthraquinones, saponins and flavonoids (Table 1). These phytochemicals are known to be biologically active and thus aid the antimicrobial activities of the plant extract. Phytochemicals exert antimicrobial activity through different mechanisms; tannins for instance act by iron deprivation, hydrogen bounding or specific interact-tions with vital proteins such as enzymes in microbial cells (Scalbert, 1991). Herbs that contain tannins as their component are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery thus exhibiting antimicrobial activity (Dharmananda, 2003). The presence of tannins in *C. occidentalis* therefore,

Table 4. Minimum inhibitory concentrations (MICs) of the DCM and NHX fractions of *C. occidentalis*.

Test organism	MIC (mg/ml)	
	DCM	NHX
Bacillus stearothermophilus (NCIB 8222)	0.313	0.157
Micrococcus luteus (NCIB 196)	0.625	0.157
Bacillus anthrax (LIO)	1.250	0.313
Enterococcus faecalis (LIO)	0.313	1.250
Clostridium sporogenes (NCIB 532)	1.250	1.250
Bacillus polymyxa (LIO)	0.157	0.157
Pseudomonas aeruginosa (NCIB 950)	0.625	0.313
Klebsiella pneumoniae (NCIB 418)	0.313	0.157
Escherichia coli (NCIB 86)	0.157	0.625

Key: DCM = dichloromethane; NHX = n-hexane; LIO = locally isolated organisms; NCIB = National Collection of Industrial Bacteria.

Table 5. Minimum bactericidal concentrations (MBCs) of the DCM and NHX fractions of *C. occidentalis*.

Test organisms	MIC (mg/ml)	
	DCM	NHX
Bacillus stearothermophilus (NCIB 8222)	0.63	0.31
Micrococcus luteus (NCIB 196)	2.50	0.31
Bacillus anthrax (LIO)	2.50	1.25
Enterococcus faecalis (LIO)	1.25	2.50
Clostridium sporogenes (NCIB 532)	2.50	5.00
Bacillus polymyxa (LIO)	0.31	0.31
Pseudomonas aeruginosa (NCIB 950)	2.50	0.63
Klebsiella pneumoniae (NCIB 418)	0.63	0.31
Escherichia coli (NCIB 86)	0.31	2.50

Key: DCM = dichloromethane; NHX = n-hexane; LIO = locally isolated organisms; NCIB = National Collection of Industrial Bacteria.

supports the traditional medicinal use of this plant in the treatment of different ailments.

Motal et al. (1985) revealed the importance of tannins for the treatment of inflamed or ulcerated tissues. Li et al. (2003) reviewed the biological activities of tannins and observed that tannins (whether total or pure compound) have remarkable activity in cancer prevention and anti-cancer. This implies that *C. occidentalis* can serve as source of drug for the treatment and prevention of cancer. In addition to its antimicrobial, anticancer activities, tannins have roles such as stable and potent antioxidants. The aforementioned observations support the use of *C. occidentalis* in herbal cure remedies. Flavonoid is another phytochemical observed in the leaf extract of *C. occidentalis*; flavonoids exhibit a wide range of biological activities which are: antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic effect and anti-oxidant properties (Hodek et al., 2002). Flavonoids ability of scavenging hydroxyl radicals, superoxide anion radicals and lipid peroxyradicals highlights many of the flavonoid health-promoting functions in organisms, which are important for prevention of diseases associated with oxidative damage of membrane, proteins and DNA (Ferguson, 2001).

Flavonoids in human diet may reduce the risk of various cancers as well as preventing menopausal symptoms (Hodek et al., 2002). All these facts supports the usefulness of *C. occidentalis* in folklore remedies and one of the reasons why this plant is widely used for the treatment of many diseases among many tribes in Africa. In addition to the antimicrobial activities exhibited by flavonoids, it also exhibits anti-trypanosomal and anti-leishmanial activities (Tasdemir et al., 2006). Epidemiological studies suggest that the consumption of flavonoids is effective in lowering the risk of coronary heart disease (Rice-Evans and Miller, 1996). Furthermore, several flavo-

noids exhibit antiviral activities (Xu et al., 2000).

Saponin which is also one of the constituents of *C. occidentalis* is responsible for numerous pharmacological properties (Estrada et al., 2000). Saponins are considered a key ingredient in traditional Chinese medicine and are responsible for most of the observed biological effects (Liu and Henkel, 2002); it is also known to produce inhibitory effects on inflammation (Just et al., 1998). Lastly, anthraquinones, another phytochemical found in *C. occidentalis*, has laxative effects which make it to be used for managing constipation (Abo et al., 2001; Sakulpanich and Gritsanapan, 2009). Anthraquinones is used in making antineoplastic which is used in the treatment of cancers; it also possess antimalarial properties (Huang et al., 2007; Leu et al., 2008; Jasril et al., 2003; Hou et al., 2009; Isabel et al., 1995). These properties confirm the uses of *C. occidentalis* in the management of cancer and malaria in folklore medicine. The observations enumerated on the properties of phytochemicals from *C. occidentalis* assert its usefulness in traditional medicines and its relevance on folklore medicine in the management of infectious and oxidative stress related diseases.

C. occidentalis is used for the preparation of decoctions among the Yoruba tribe of South Western Nigeria for consumption in the treatment of ailments, thus indicating that the plant extract is non-toxic. Hence, different formulations from this plant for healthcare delivery may be safe for consumption.

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