Research Article

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Antibacterial Activity of *Cassia occidentalis* Flower Vegetable Extract on Selected Bacteria.

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Abstract

The development of drug resistance as well as appearance of undesirable side effects of certain antibiotics has lead to the search for new antimicrobial agents mainly from plant extracts with the goal to discover new chemical structures, to overcome these disadvantages. The research was therefore carried out to evaluate the in-vitro antibacterial activity of *Cassia occidentalis flower* extract. The clinical bacterial isolates, *Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae* and *Pseudomonas aeruginosa* were subjected to antibacterial susceptibility test using agar well diffusion method. The results showed that all the extracts had activity against *Klebsiela pneumoniae* at a concentration between 30 – 90mg/ml. There was no antimicrobial activity exhibited against the other three test organisms. The Minimum Inhibitory Concentration ranged between 35 – 55mg/ml for water extract and 25 – 55mg/ml for chloroform extract. The Minimum Bactericidal Concentration was 55 mg/ml by both water and chlorofrom extract . The phytochemical analysis of the flower extracts revealed the presence of tannin, flavonoid, anthroquinone, saponin, carbohydrates, and cardiac glycoside. *C. occidentalis* flower extract might therefore be used to treat *Klebsiella* associated illness such as pneumonia and bronchitis.

Keywords: *Cassia occidentalis*, Antibacterial activity, Flower, Phytochemicals.

Introduction

For the past two decades, there has been an increasing interest in the investigation of different extract obtained from plants as a source of new antimicrobial agents [1] Medicinal plants are plants in which one or more of its organs contain substances that can be used for therapeutic purposes [2]. Majority of rural dwellers do not have access to modern health care, so the mostly depend on medicinal plants to prevent or eliminate diseases ([3].

Occidentalis species belongs to the genus *Cassia* and the Family *Caesalpiniaceae*. It is called Stinking Weed,. In Hausa, it is known as "Rai dore" . In Igbo it is called "Osiisi" while ''Gaya'' in Nupe. It is an erect herb, commonly found by road sides, ditches and waste dumping sites. *Cassia occidentalis* has many applications in traditional medicine. All the parts of the plant have medicinal uses. Tradionally, its roots leaves, flowers and seeds are used as laxatives and purgative [4]. Phytochemically, the aqueous extract of Cassia occidentalis contained tannins, anthraquinone, sterol, cardiac glycosides, saponin and alkaloids [5].

Previous works have shown that C. occidentalis leaves exhibited *In vitro* antibacterial, antimalarial and antihepatoxic property [6]. The seeds are brewed into a coffee like beverage for asthma and flower infusion is use for the treatment of bronchitis in the Peruvian amazon [7]. Although the flower infusion was reported to be used locally for the treatment of bronchitis in the Peruvian amazon, literature survey revealed that the flower extract has yet not been screened for its antimicrobial activity. Therefore the study was carried out to determine the phytochemical and antimicrobial activities of *C. occidentalis* flower extract on selected bacteria namely, *Klebsiella pneumoniaea, Staphylococcus aureus Streptococcus pneumoniae, Pseudomonas aeruginosa.*

Materials and Methods

Collection and Identification of Plant material

The fresh flower of *Cassia occidentalis* was collected within Minna town and was identified and authenticated by Mr. Daoud Oladipupo of Department of Biological Science Federal University Technology Minna Niger state.

Preparation of the plant material

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The flowers were sun dried for 1 week until a constant weight were obtained and grounded into powder using mortar and pestle. An electric blender (National Mx 391N, Matsuhita electric) was then use to micronise the sample into fine powder to ensure proper penetration of the extracting solvent into the cell to facilitate the release of the flower's active ingredients.

Extraction

Fifty grammes of the powdered flower specimen was weighed and extracted with 500ml of distilled water and chloroform. The water extraction was for 24 h at 4^0C with occasional shaking, while chloroform extraction was for 24h at room (28 ± 2^0C) temperature. Each mixture was filtered and the filtrate was evaporated to dryness in an evaporating dish on a steam bath at a temperature of 70^oC. These extracts were stored in screw-capped bottles and kept in the laboratory refrigerator for further research.

Phytochemical screening of the plant extract

Basic/preliminary phytochemical screening which consists of performing simple chemical tests to detect the presence of tannins, alkaloid, anthranoids, saponins, anthraquinone, glycosides, etc. was performed in the department of Biochemistry of Federal University of Technology, Minna following the methods of [8] and [9].

Reconstitution of the Extract

Each dry extract was reconstituted in to 300mg, 500mg, 700mg and 900mg in 5ml of distilled water. For chloroform extract, 10% of the solvent was used to dissolve the extract before 90% sterile water was added to make up 100%

Test microorganisms

Four bacterial isolates were obtained from the General Hospital Minna. These included two gram-positive bacteria (*Staphylococcus aureus and Streptococcus pneumoniae)* and two gram-negative bacteria (*Pseudomonas aeruginosa* and *Klebsiella pneumoniae)*.

Identification of test microoganisms

Gram stain procedure as well as series of biochemical tests such as haemolysis, catalase, indole, citrate, coagulase, oxidase and urease test was carried out to confirm the purity of the isolate before transferred into slant and incubated at 35^0C for 24 hours. The reference bacteria were maintained on Nutrient agar slants at 4^0C before subculture.

Inoculum preparation

A loopful of isolated colonies was inoculated into 5ml broth and incubated at 37^0C for 18 hours. The turbidity of actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5 McFarland units prepared by mixing 0.5 ml of 1.75% (w/v) Barium chloride dehydrate with 99.5 ml 1% (v/v) Sulphuric acid. This turbidity was equivalent to approximately 1×10^6 colony-forming units per milliliter (cfu/ml).

Antimicrobial bioassay

The antimicrobial activities of the extracts were determined by agar well method according to [10]. Nutrient Agar was used for the antimicrobial activity test. Nutrient agar medium was prepared, sterilized and under aseptic conditions dispensed into pre-sterilized petri-dishes to yield a uniform depth of 4 mm after being inoculated by 0.5ml standardized bacterial culture, respectively. Sterile cork borer (4mm diameter) was used to bore wells on each plate. Each hole was filled with 3 drops of the extract using Pasture pipette and was allowed to stand for 15 minutes for proper diffusion of the extract to take place into the media. Positive antibiotics contro (Chloramphenicol) was also prepared. The plates were allowed to stay for 15 minutes for maximum diffusion of the extract into the media to take place and incubated at 37^0C for 24 hours. The diameter of zone of inhibition as indicated by clear area which was devoid of growth of microbes was measured.

Determination of minimum inhibitory concentration by tube dilution method

The MIC of the antimicrobial compound was determined by broth dilution method. Nutrient broth test tubes were prepared and labeled. In all the test tubes containing 5ml of sterile broth, 0.5ml of bacteria suspension (1.0×10^6) was inoculated. This was followed by the addition of serial concentrations (5mg/ml, 15mg/ml, 25mg/ml, 35mg/ml, 45mg/ml, and 55mg/ml) of the flower

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extract to the sterile nutrient broth test tubes. In the control tubes, the test antimicrobial compound was not added. The un-inoculated test tube was used to check the sterility of the medium and as negative control while the positive control tube was used to check the suitability of the medium for growth of the microorganisms and the viability of the inoculums. The final volume in all the test tubes was adjusted to 10ml using sterile water. All the test tubes were properly shaken and then incubated at 37^0C for 24 hours and the change(s) in turbidity was observed. The MIC was determined by the lowest concentration of the flower extract that prevented visible growth.

MBC was determined by sub-culturing from every tube that has shown no growth. The test tubes that have shown no detectable turbidity were streaked on the surface of nutrient agar plates and incubated at 37^0C for 24 hours. The minimal concentration of extract that has no growth was detected on sub-culturing was taken as the MBC.

Results

Solvent used	Yield in grammes
Water	10
Chloroform	07

Table 1: Extractive quantity of the flower in different solvents

Plant extract	Colour	Texture	Odour
Water	Brownish	Sticky, oily	Stout beer
Chloroform	Dark black	Powdery	Slightly pungent

Table 2. Organoleptic property of the Cassia occidentalis flower extract

Plant chemicals	Methods	Water extracts	Chloroform extracts
Alkaloids	General	-	-
Tannin	General	++	++
Flavonoid	General	+	++
Anthraquinone	General	+++	+++
Saponin	Frothing	+++	+++
Polyphynol	General	-	+
Carbohydrate	General	+	+
Cardiac glycosides	General	+	++
Sesquiterpene	General	-	-

Table 3. Phytochemical analysis of water and chloroform extract of Cassia occidentalis flower

KEY

+++ = Highly present, ++ = moderately present

+ = trace amount, - = completely absent

Test bacterial isolates	Concentration in mg/ml	DZI in mm mean±SD) By water extract	DZI in mm (mean ±SD) by antibiotic chloramphenicol
Klebsiella pneumoniae	30	5.5±0.5	16.5±0.5
	50	9.5±0.5	23±1
	70	12±1.0	24.5±0.5
	90	16.5±0.5	26.5±0.5
Staphyloccus aureus	30	0	7.5±0.5
	50	0	10.5±0.5
	70	0	12±1
	90	0	15.5±0.5
Streptococcus pneumoniae	30	0	11±1
	50	0	13.5±0.5
	70	0	15±1
	90	0	19.5±0.5
Pseudomonas aeruginosa	30	0	3.5±0.5
	50	0	5.5±0.5
	70	0	8±1
	90	0	13±1

Table 4. Antibacterial activity of water extract on the test bacteria
Key: DZI = Diameter of zone of inhibition; SD =Standard Deviation

Organism Control	Water extract	Chloroform extract
Klebsiella pneumoniae -	35.00	25.00

Table 5. Minimum inhibitory concentration (MIC) of extracts on test organisms.

MIC value in mg/ml

MBC value in mg/ml

Organism	Water extract	Chloroform extract	Control
Klebsiella pneumoniae	55.0	55.0	-

Table 6. Minimum bactericidal concentration of extract on the test organism

Discussion

The extraction of flower of *Cassia occidentalis* yield 10.0g from water and 7.0g from chloroform. The difference in the quantity yield may be attributed to the difference in the polarity of the two solvent used (ie. Chloroform < water). The color, texture and odor of the flower extracts in different solvents in dried conditions differ remarkedly. The preliminary phytochemical analysis of the extract revealed the presence of tannin, flavonoid, anthroquinone, saponin, carbohydrate, and cardiac glucoside. These compounds have been reported to inhibit bacterial growth [11]. There is slight difference in the phytochemical constituent of the two extract: flavonoid and cardiac glycoside were in trace amount in water extract while moderately present in chloroform extract. This might be due to the fact that water is a poor extracting solvent for plant chemicals. *C. occidentalis* have been used by local people for the treatment of bronchitis in the Peruvian amazon as reported by [12]. The results of the present study agreed essentially with the report of these previous investigators because it revealed activities against *K. pneumonia* which is one of causative agent of pneumonia and bronchitis. Lack of antibacterial activities exhibited by the extracts of *Cassia occidentalis* against the other three test bacteria (*S. aureus, S. pneumoniae* and *P. aeruginosa)*, generally at concentrations between 30 – 90mg/ml is suggestive of limited antibacterial activity of the flower extract. The antibacterial activity exhibited by water and chloroform flower vegetable extracts of *Cassia occidentalis* on *K. pneumoniae* agreed with the report of [13], that all parts of *C. occidentalis* has medicinal uses. The result also agreed with the fact that the minimum concentration of antimicrobial agent required to kill microorganisms can be equal or greater than the minimum inhibitory concentration for that microbes [14].

Overall, the present study indicates the antimicrobial properties of flower extract of *C. occidentalis* and provides some idea about the extract phytochemical constituents. The flower extract has no activity against *Staphylococcus aureus, Streptococcus pneumoniae,* and *Pseudomonas aeruginosa.* In the case of *Klebsiella pneumoniae* the extract recorded appreciable activity. It is also a clear revelation that the flower extract of *Cassia occidentalis* can be used to treat *Klebsiella* associated ailment such as pneumonia, bronchitis and other diseases known to cause by *K. pneumonia.* It is however recommended that further attention and research should be conducted to identify the active compound responsible for the antimicrobial activity.

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