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Phytochemical screening and antibacterial activities of cassia fistula leaf extracts on some selected pathogens

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Abstract
Cassia fistula, commonly known as golden shower tree has for long been used for treatment of number of ailment as decoction from its leaves by many. This study was carried out with the aim to evaluate the antibacterial activity and investigate the phytochemical properties of the leaves of *Cassia fistula* plant. Hot water, cold water and ethanol extracts of leaves of *Cassia fistula* were examined against medically important bacteria (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Salmonella typhi*) by agar disc diffusion method using different extracts concentration. There was a remarkable inhibition of the bacterial growth against all tested organisms with highest activity seen at 100µg/ml for all the hot water, cold water and ethanol extract. Phytochemical analysis was carried out to determine the phytochemical constituents present in the leaf. Phytochemical screening revealed the presence of capric acid, palmitic acid, oleic acid, 1, E-11, Z-13-octadriene, 9-octadecenal, brassidic acid. The result from this study is an indication that *Cassia fistula* leaf is effective against these pathogens and can be further exploited for the development of new antimicrobials.

Keywords: *Cassia fistula*, phytochemicals, antibacterial effect, sensitivity

Introduction
Antibiotics have saved the lives of millions of people and have contributed to the improved life expectancy of many. However, with the emergence of Multi-drug resistant (MDR) pathogens, the clinical efficacy of many antibiotics is becoming threatened [1]. Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health threat as there are fewer, or even sometimes no effective antimicrobial agents available for infections caused by these resistant bacteria. These reasons make the search for alternative medicinal plants as antimicrobial agents of paramount importance, because they possess a variety of chemical constituent in nature that are known to cure many ailments. One of such plant is *Cassia fistula*, commonly known as golden shower tree of the family caesalpinaceae [2]. It is an important medicinal plant that is native to India, the Amazon and Sri Lanka and diffuses in various countries including Mexico, Mauritius, China, South Africa and West Africa [3]. It is a deciduous medium sized tree growing up to 20 to 40 meters in height. The bark of this plant is rough grayish and leaves are pinnately compound. It has showy racemes, up to 40 cm long with bright yellow fragrant flowers. Fruits of these plants are long and cylindrical pod with seeds that broadly ovate and horizontally arranged in the sweetish pulps [4].
Medicinally, the plant is known to have various pharmacological activities including anti-bacterial [5], anti-fungal [6], anti-inflammatory [7], anti-oxidant [8], anti-tumor [9].
The traditional uses of plants range from the administration of the roots, barks, stems, leaves and seeds, to the use of extracts and decoction from the plants [10].
There is an urgent need to investigate these plant parts in a search for alternative antimicrobial drugs to stem the tide of bacteria resistance and explore alternative source for the treatment of infectious diseases.

Materials and Methods
Collection and maintenance of organisms used
The test organisms used were all human pathogenic organisms of clinical origin. These isolates include; *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* and *Klebsiella pneumoniae*; they were obtained from the Microbiology laboratory of Bells University of Technology, Ota.

Pure cultures were obtained by culturing the bacterial isolates on their selective media and incubating at 37°C for 24 hours, after which they were inoculated on Nutrient agar slants, incubated at 37°C for 24 hours and kept as stock cultures in the refrigerator at 4°C before utilization. Biochemical tests were performed on each of the test organism to confirm their identities.

Collection of plant materials

Leaves of *Cassia fistula* were collected from Covenant University, Ota Ogun State Nigeria and were authenticated by experts. The collected samples were thoroughly washed with tap water and then air dried. Properly dried leaves and flowers were grinded until turn into fine powders and stored in air tight bottles.

Extraction of plant materials

20g of the grinded samples of leaves was extracted in 200ml cold distilled water, hot water and ethanol for cold distilled water, hot water and ethanol extract respectively for 72 hours and was then filtered through Whatman No.1 filter paper into a sterile conical flask. Finally, these filtrates were evaporated by using water bath leaving behind the extract (residue). The residue of the extract was then measured on a weighing balance and then kept in refrigerator for the mean time before usage.

Preparation of extract concentrations

Different concentration of 100, 50, 25, 10, and 5 µg/ml concentrations of ethanol extract, hot water extract and cold water extract were prepared in sterile McCartney bottle by weighing 100µg, 50µg, 25µg, 10µg and 5µg of each of the extract, 1ml of tween20 was then added to each of the weighed extract. The prepared extracts were then covered with foil paper.

Preparation of 0.5 Mcfarland standard turbidity solutions

A 0.5 McFarland standard turbidity solution was prepared by adding 0.5g of $BaCl_2$ to 50ml of distilled water. In a separate tube, 99ml of distilled water was added to 1ml of H_2SO_4 (1% v/v). The two solutions were mixed thoroughly to ensure an even suspension. 0.5 of the H_2SO_4 solution was removed and discarded; 0.5ml of the $BaCl_2$ solution was taken and added to the H_2SO_4 solution to make the McFarland standard. The 0.5 McFarland standard turbidity solutions correspond to a homogenous *Escherichia coli* suspension of 1.5×10^8 cells/ml [11].

Antibacterial activity; agar diffusion test

The antibacterial activity of various extracts (ethanol, cold water and hot water extract) of *Cassia fistula* was determined by using agar well diffusion method. The selected bacterial cell suspensions were prepared in McCartney bottles separately, Turbidity of the inoculums of various bacterial isolates were compared with 0.5 McFarland standard and each of the isolates was spread evenly on the surface of prepared Mueller Hinton agar plates using a sterile cotton swab until uniform distribution of the inoculum was achieved. About 8mm diameter wells were made by using sterilized cork borer. The tests were performed using 5 different concentration of the *Cassia fistula* extract (100, 50, 25, 10, and 5µg/ml). Distilled water (hot and cold water), and ethanol served as negative control. The plates were incubated for 24 hours at 37^0C in the incubator. After which zone of inhibition was measured and recorded in millimeters.

Antibiotic Susceptibility test

Antibiotic susceptibility test was carried out on each of the pathogenic isolates as positive control (either drug resistant or sensitive). Multi-sensitivity discs bearing eight different antibiotics; Cefazidime (30µg), Cefuroxime (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Ofloxacin (5µg), Augmentin (50µg), Nitrofurantonin (300µg), Ampicillin (10µg) were aseptically placed with the aid of sterile forceps over the surface of Mueller Hinton which had been previously streaked with the pathogenic test bacterial suspensions with prescribed turbidity (compared to that of 0.5 McFarland standard). The plates were incubated at 37°C for 24hours. After incubation, the diameter of the zone of inhibition around each well was measured to the nearest millimeter.

GC-MS analysis

Phytochemical screening was carried out in order to test for the presence of chemical constituents of the cassia fistula leave extract. For the identification of chemical composition of the *cassia fistula* extract, the analysis was performed on a GC-MS-Q P2010 plus Shimadzu model equipped with a Mass Spectrometer detector. The column used was RTX-1-Integral and contained (5%-diphenyl-95%-dimethylpolysiloxane). The column dimension was 30m×0.25mm and the column oven was programmed at 70°C ramped at 10°C/min up to 280°C at 5 min. The injection temperature was 250°C, the flow rate of the helium gas was 1.80mL/min and the film thickness was 0.25µm. Compounds were identified by means of the NIST library.

Results

The results of the antibacterial effect of the different concentrations of the aqueous and ethanolic leaf extracts showed that all extracts were effective with measurable zones of inhibition against the selected human pathogens represented in Table 1 to 3. Ethanolic leaf extract was most effective amongst all three extracts with a zone of inhibition ranging from 10mm to 36mm at the lowest concentration of 5µg/ml to 10µg/ml as shown in Table 1.

The hot water leaf extract also showed a remarkable zone of inhibition amongst all test Organisms (Table 2).

The least efficacy amongst all three extracts was observed in cold water leaf extract which had the least zone of inhibition amongst all test organisms with the lowest zone of inhibition of 8mm at a concentration of 5µg/ml and at 20mm at 100µg/ml for *Salmonella typhi* (Table 3).

Antibiotics susceptibility test performed demonstrated that all the test bacteria were inhibited by at least one antibiotic except for *Pseudomonas aeruginosa which* was resistant to all eight antibiotics used in this study and *Escherichia coli* also showed sensitivity to Gentamicin with an inhibition zone of 20mm at 10 10µg and was resistant to all other antibiotics (Table 5). *Salmonella typhi and Staphylococcus aureus* was however susceptible to four antibiotics and resistant to others while *Klebsiella pneumoniae* was susceptible to Ceftazidime, Cefuroxime and Ciprofloxacin and resistant to all other five (Table 5).

The GC-MS analyses of the *Cassia fistula* extract revealed that the leaf contained 18 compounds representing 99.5% of the total composition and the major compounds identified were wereoleic acid (38.6%), 1, E-11, Z-13-octadecatriene (19.0%), palmitic acid (16.0%) and stearic acid (11.3%) as represented in Table 6.

Table 1: Antibacterial activities of ethanol extracts of leaves of *Cassia fistula* against bacterial test organisms

Microorganisms	Concentration in µg/ml				
	5	10	25	50	100
	Zone of inhibition in mm				
Escherichia coli	24	26	26	30	36
Klebsiella pneumonia	16	17	20	25	30
Pseudomonas aeruginosa	20	22	24	26	30
Salmonella typhi	16	18	20	24	28
Staphylococcus aureus	10	10	14	16	18

Table 2: Antibacterial activities of hot water extracts of leaves of *Cassia fistula* against bacterial test organisms

Microorganisms	Concentration in µg/ml				
	5	10	25	50	100
	Zone of inhibition in mm				
Escherichia coli	20	20	26	28	30
Klebsiella pneumonia	18	20	20	24	38
Pseudomonas aeruginosa	20	20	24	26	26
Salmonella typhi	20	21	22	24	28
Staphylococcus aureus	16	18	24	26	36

Table 3: Antibacterial activities of cold water extracts of leaves of *Cassia fistula* against bacterial test organisms

Microorganisms	Concentration in µ/ml				
	5	10	25	50	100
	Zone of inhibition in mm				
Escherichia coli	18	20	20	28	30
Klebsiella pneumonia	18	20	22	22	28
Pseudomonas aeruginosa	20	20	24	28	29
Salmonella typhi	08	12	14	16	20
Staphylococcus aureus	14	18	20	24	26

Table 5: Antibiotics susceptibility of the test organisms

Isolates	CAZ (30µg)	CRX (30µg)	GEN (10µg)	CPR (5µg)	OFL (5µg)	AUG (30µg)	NIT (300µg)		AMP (10µg)
Escherichia coli	R	R	20 (S)	R	2 (I)	R	R		R
Klebsiella pneumonia	26 (S)	R	20 (S)	26 (S)	20		R	14 (R)	R
Pseudomonas aeruginosa	R	R	14(I)	16 (I)	12 (R)		R	R	R
Salmonella typhi	28 (S)	30 (S)	26 (S)	R	24 (S)		R	R	R
Staphylococcus aureus	R	R	20 (S)	28 (S)	22 (S)		R	26 (S)	R

KEY: CAZ-Cefazidime, CRX- Cefuroxime, GEN-Gentamicin, CPR-Ciprofloxacin, OFL-Ofloxacin, AUG- Augmentin, NIT- Nitrofurantoin, AMP- Ampicillin, S- Susceptible, R- Resistance, I- Intermediate.

Table 6: Chemical Composition of the Cassia fistula Extract

Compound	Retention Index	Percentage Composition
capric acid methyl ester	1282	0.2
undecylenic acid	1461	2.0
hendecanoic acid	1471	1.0
lauric acid	1570	1.0
n-tridecanoic acid methyl ester	1580	0.2
n-tetradecoic acid	1769	1.4
methyl-14-methylpentadecanoate	1814	0.2
1,E-11,Z-13-octadecatriene	1817	19.0
palmitic acid, methyl ester	1878	0.2
palmitic acid	1968	16.0
9-octadecenal	2007	0.8
methyl-11-octadecenoate	2085	1.0
Methyllinolelaidate	2093	0.5
stearic acid	2167	11.3
oleic acid	2175	38.6
arachidic acid	2366	1.0
α-monopalmitin	2482	1.8
brassidic acid	2572	3.3
Percentage Total		99.5

Discussion

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. This study was conducted with a view to investigate the antimicrobial properties of *Cassia fistula* extracts against

some human clinical bacterial isolates (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococcus aureus*). All extracts showed significant zones of inhibition against the five bacterial test isolates. The results show a remarkable inhibition of the bacterial growth against the tested organisms with highest activity seen at 100µg/ml for all the hot water, cold water and ethanol extract. *Escherichia coli* have shown an average highest activity while *Staphylococcus aureus* have shown an average lowest activity. Ethanol extract showed the highest inhibitory effect on all organisms except on *Staphylococcus aureus* where hot water extract showed the highest inhibitory effect and *Salmonella typhi* where the inhibitory effect of hot water extract was closely the same as the ethanol extract. Sensitivity of test organisms to the different extracts in descending order, from most sensitive to least sensitive is demonstrated below:

Ethanol extract: *Escherichia coli* ≥ *Pseudomonas aeruginosa* ≥ *Klebsiella pneumonia* ≥ *Salmonella typhi* ≥ *Staphylococcus aureus.*

Hot water extract: *Staphylococcus aureus* ≥ *Escherichia coli* ≥ *Salmonella typhi* ≥ *Klebsiella pneumonia* ≥ *Pseudomonas aeruginosa.*

Cold water extract: *Escherichia coli* ≥ *Pseudomonas aeruginosa* ≥ *Klebsiella pneumonia* ≥ *Staphylococcus aureus* ≥ *Salmonella typhi.*

Susceptibility of the test organisms to the different antibiotics showed that the test organisms were inhibited by at least one antibiotic except for *Pseudomonas aeruginosa which* was resistant to all the antibiotics. *Salmonella typhi and Staphylococcus aureus* was however susceptible to four antibiotics, while *Klebsiella pneumoniae* was susceptible to three and *Escherichia coli* was susceptible to one.

These results obtained from this study shows that the plant *Cassia fistula* plant extracts is inhibitory against the bacterial test organism used in this study compared with standard antibiotics used as has been reported by other researchers.

This infers that *Cassia fistula* is effective against most of the clinically isolated bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aureus* and thus conforms to the work of Nayan and Shukla, 2011; Seyyed *et al*., 2014 and in disparity with the work of Aneja *et al.*, 2011 who discovered that ethanolic extract of *Cassia fistula* leaves has no inhibitory effect on *Pseudomona aeruginosa.*

Although the test organisms involved in this research work involved more Gram negative than Gram positive bacteria, in 2006 Jigna and Sumitra, have reported stronger activity of plant extracts against Gram-positive bacteria than that of Gram negative bacteria. The difference in efficacy of different extract maybe because different solvents have different polarities, hence different degrees of solubility for the various phytoconstituents [16].

The phytochemical analysis of the plants shows that the microbial activity of the *Cassia fistula* was due to the presence of various secondary metabolites that were proved to be present in the *cassia fistula*. Hence, these plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities.

The findings from the sensitivity test also showed high resistance pattern of these organisms to synthetic antibiotics as shown in table 4 in comparison to the high inhibitory effects of plant extracts against these organisms, the results may henceforth be very significant because of the possibility of developing therapeutic substances that may be more active against multidrug-resistant organisms.

Conclusion

The findings in this study support the traditional therapeutic claims that this herb *Cassia fistula* is used in the treatment of ailments caused by microbes and an important source of naturally occurring bioactive compounds. Demonstration of antimicrobial activity against bacterial test organisms is an indication that the plant is a potential source for production of drugs with a broad spectrum of activity and Polyphenolics abundantly present in the extracts of the plant may prove to be very important, non-toxic chemo-preventive agents against various bacterial pathogens.

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