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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *Cassia obtusifolia* LEAF EXTRACTS

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ABSTRACT
Cassia obtusifolia is a plant which plays an important role in the traditional system of holistic health and herbal medicine in Africa. In this study phytochemical constituents and antimicrobial activity of Cassia obtusifolia leaf extracts were investigated. The leaf powder of Cassia obtusifolia was macerated with methanol and then fractionated with petroleum ether, dichloro methane and ethyl acetate. The extract and fractions were subjected to phytochemical analysis to detect the presence of secondary metabolites by using standard procedures. The extract and fractions were further subjected to antimicrobial activity against ten microorganisms namely Staphylococcus aureus, Escherichia coli, Aspergillus flavus, Aspergillus niger, Klebsiella pneumoniae, Streptococcus pyogenes, Proteus mirabilis, Salmonella typhi, Shigella dysentriae and Pseudomonas aeruginosa. The result of phytochemical analysis indicated the presence of saponins, tannins, steroids, flavonoids, glycosides, triterpenoids and alkaloids. The antimicrobial susceptibility study showed dose-dependent pattern. The ethyl acetate extract had the highest activity with the zone of inhibition of 20mm against Staphylococcus aureus at a concentration of 4000 µg /mL and a minimum inhibitory concentration at 1000 µg/ml, followed by dichloromethane extract which had zone of inhibition of 19mm against E.coli. Still at same concentration an inhibition zone of 18mm was recorded against E.coli, S.dysenteriae and S.typhi from the ethyl acetate and methanol extracts respectively. The lowest zone of inhibition (9mm) at that concentration recorded against Aspergillus niger was from methanol extract. This study showed interesting correlation between the phytochemical and biological activities hence, findings from this study, justifies the traditional use of the plant in treatment of various diseases.

INTRODUCTION

Traditional medicine also known as complementary medicine is considered the mainstay of health care delivery or its complement in most parts of the world (WHO, 2013). Humans had used this form of healthcare from time immemorial. Medicinal plants are plants which contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of new drugs. The use of natural products as medicinal agents is a tradition which dates back to prehistory. Medicinal plants over the years have played important roles in the treatment of several diseases worldwide (Fallah-Hoseini *et al*, 2006). The therapeutic value of these plants is largely dependent upon their phytochemical constituents. In Nigeria almost all plants have medicinal values and medicinal plants are considered to have intrinsic powers, hence, thought to possess inherent curing properties. Medical herbalism has become a viable profession in Nigeria such that some people in rural areas have knowledge of a wide variety of herbs that can be used to treat common ailments (Banjo *et al*., 2004).*Cassia obtusifolia* popularly called sickle pod is a branched annual herb with alternate leaves pinnately compound divided without a conspicuous gland at the base of the leaves stalk (Crowhurt, 1972). Sickle pod is very variable in its habit and can be an annual or perennial herb, or a shrub, growing up to 2 metres tall (Crowhurt, 1972). The plant is utilized locally for a variety of reasons, particularly as food and medicine, and it is occasionally cultivated for these purposes. The flowers are decorative and the plant is commonly planted as an ornamental near town. *Cassia obtusifolia* is reported to have good medicinal values in traditional system of medicine (Falade *et al.,* 2004), the leaves are anthelmintic, antipyretic diuretic and laxative.

They are also known to be use in the treatment of asthma and bronchitis, fruit and seeds of the plant are anthelmintic and also are used to cure tumours (Falade *et al.,* 2004). The development of effective and less toxic antimicrobial agents is required for the treatment of infectious diseases especially in the face of rising antimicrobial resistance This study was aimed at evaluating the antimicrobial activity of the plant against some bacterial infection by extracting the leaves using different solvents, detecting the presence of secondary metabolites and screening the extracts for antibacterial and antifungal activity against clinical isolates.

MATERIALS AND METHODS

Collection and preparation of plant

Fresh leaves of *Cassia obtusifolia* was collected from a farm in Dutse local government area, Jigawa State and identified in the Department of Botany, Federal University Dutse, Jigawa State. The fresh leaves were air-dried, ground into powdered and stored at room temperature for further experiment.

Maceration of plant material

Five hundred grams (500g) of the air dried powdered leaves were soaked in a 2 litre conical flask containing 95% methanol (1.5L) stoppered and kept for two weeks with occasional shaking and stirring, then the mixture was decanted and filtered and the filtrate was evaporated to dryness by using rotary evaporator (RE 50rpm) at 40^0C. Warm water was added to the concentrated methanol extracts and stirred, the water soluble portion was decanted, this was repeated severally for exhaustive extraction, then petroleum ether was added to the water soluble portion and mixed thoroughly in a seperatory funnel and allowed to settle after which the lower layer was gently drained and the upper layer was collected, concentrated, dried and labelled as the petroleum ether fraction. The same procedure was repeated to the remaining water soluble portion using dichloromethane then ethyl acetate, each fraction was concentrated, dried, weighed and labelled (Momoh *et al.,* 2017).

Preliminary phytochemical screening

The Phytochemical Screening of the leaves extracts of *cassia obtusifolia* plant were performed using standard methods as described by Akroum *et al.* (2017), Trease and Evans (2005) and Sofowora (1993). Secondary metabolites tested include tannins, flavonoids, alkaloids, saponins, and steroids.

Anthraquinone test

Borntrager's test: To 3ml of the extracts, 1 ml dilute H_2SO_4 was added. The solution was then boiled and filtered using Whatman filter paper. The filtrate was cooled to room temperature and to it equal volume of benzene was added. The solution was shaken well and the organic layer was separated. Equal volume of dilute ammonia solution was added to the organic layer. The ammonia layer tuned pink showing the presence of anthraquinone glycosides (Sofowora,1993).

Ferric chloride test for Phenols

To 1ml of the extracts, 2ml of distilled water was added followed by few drops of 10% ferric chloride. Appearance of blue or green colour indicates the presence of phenols (Trease and Evans 2005).

Flavonoid test

Three different tests were used for the identification of flavonoids.

(i) Potassium hydroxide (KOH) test: 1 ml of each extract was treated with few drops of 10% potassium hydroxide solution. Formation of intense yellow colour indicates the presence of flavonoids (Trease and Evans, 2005).

(ii) Alkaline test: 0.5 ml of extract was treated with few drops of 1M sodium hydroxide solution. Formation of intense yellow colour which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids (Trease and Evans, 2005).

(iii) **Lead acetate test**: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids (Trease and Evans, 2005).

Ferric chloride test for Tannins

0.5 ml of the extract was boiled with 10 ml of distilled water in a test tube and then, few drops of 5% ferric chloride solution was added and the reaction mixture was observed for greenish black colour change which was indicative of a positive test (Sofowora ,1993).

Alkaloid test

Two different tests were used for the identification of alkaloids. Small quantity of the extract was stirred with 5ml of 1% aqueous hydrochloric acid on a water bath and filtered. 2ml of the filtrate was divided into two portions.

(i) **Dragendroff's test:** one portion of the filtrates was treated with Dragendroff"s reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids (Silva *et al*., 1998).

(ii)Wagner's test: To 0.5 ml of the extract, 2 ml of Wagner"s reagent (Iodine solution 5%) was added and the reaction mixture was observed for the formation of reddish-brown precipitate (Silva *et al*., 1998).

Test for Saponins

Frothing test: 0.5 ml of the extract was added to 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for

the stable persistent froth. (Trease and Evans 2005).

Carboxylic acid test

1 ml of the various extracts was separately treated with a few ml of 1M sodium bicarbonate solution. Effervescence (due to liberation of carbon dioxide) indicates the presence of carboxylic acid (Harborne,1973)

Glycoside test

5ml each of various extract were hydrolyzed separately with 5 ml each of conc. HCl and boiled for few hours on a water bath and hydrolysates were subjected to the following test: A small amount of alcoholic extract of samples was dissolved in 1 ml water and then aqueous 10% sodium hydroxide was added. Formation of a yellow colour indicated the presence of glycosides (Harborne, 1973).

ANTIMICROBIAL ACTIVITY

Test isolates

The organisms used in this work were *Staphylococcus aureus, Escherichia coli, Aspergillus flavus, Aspergillus niger, Klebsiella pneumonia, Streptococcus pyogenes, Proteus mirabilis, Salmonella typhi, Shigella dysentriae and Pseudomonas aeruginosa.*The test organisms were clinical isolate obtained from Microbiology laboratory of Aminu Kano Teaching Hospital, (AKTH) Kano State Nigeria and they were maintained on nutrients agar slants in the refrigerator (4^{o}C) before use.

Preparation of extracts

Stock solutions of extracts were prepared by weighing 8mg of each fraction and dissolving in 1ml dimethyl-sulfoxide (DMSO) in a sterilized bijou bottles and the mixture was shaken thoroughly to dissolve the solution giving a concentration of 8000µg/ml.

The concentrations were prepared by serial doubling dilution method of extracts or fractions. 0.5ml of each of the various stock solutions was weighed and mixed with 0.5ml of DMSO to give the concentrations 4000µg/ml, 2000µg/ml, 1000µg/ml and 500µg/ml respectively. The standard control (ciprofloxacin and Nystatin) for bacteria and fungi were also prepared by dissolving 0.5ml of each drug in 0.5ml distilled water.

Antimicrobial Bioassay

The media used in this work were nutrient agar and potato dextrose agar. The media were prepared as described or instructed by the manufacturers and distributed in to petri dishes to cool and solidify. A loopful of the test isolate was picked using a sterile wire loop and emulsified in 3 to 4 mililitres of sterile physiological saline followed by proper shaking. The turbidity of the suspension was matched with that of 0.5 McFarland (Cheesbrough, 2000).

Sensitivity testing of the extracts

Standard inocula of each isolate were swabbed onto the surface of nutrient agar in separate petri dishes of the extract and standard antibiotic placed. Five wells were cut in each plates by using 6mm in diameter sterile cork borer, an aliquot of 100µl of the various concentrations of the extracts was added into the wells using dropper. The plates were inverted and allowed to stand for 30mins for the extracts to diffuse into the agar after which the plates were incubated aerobically at 35^{0}c for 18 hours. This was followed by measurement of zone of inhibition of extract and the standard antibiotic against the test organisms in mm by the use of transparent ruler.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MIC) were determined for the extracts using nutrient broth dilution method in accordance with Clinical and Laboratory Standard Institute (CLSI) (2015). Nutrient broth was prepared according to the manufacturer's instruction. Mc –farlands turbidity scale was prepared to give turbid solution. Normal saline was prepared and dispensed into test tubes and the microorganisms were inoculated and incubated at 37^{o} C. Dilution of the test microorganisms in the normal saline was performed until the turbidity marched that of the Mac farlands scale by visual comparison. Two fold serial dilutions of the extracts in the broth were performed to obtain the concentration of 4000µg/ml, 2000µg /ml, 1000µg /mL, and 500 µg /ml for the extracts. 0.1 ml of the standard inoculum of the test microorganism in the normal saline was then inoculated into the different concentrations of the extracts. Incubation was made for 24 hours after which each broth was observed for turbidity. The lowest concentration of the extract in the broth which showed no turbidity was recorded as the minimum inhibitory concentration (MIC) (Garba *et al.*, 2019).

Minimum bactericidal concentration and fungicidal concentration (MBC/MFC). The minimum bactericidal concentration / minimum fungicidal concentration (MBC/MFC) were determined according to Bauer *et al.* (1966). The content of the MIC in the serial dilution was sub- cultured onto the prepared medium and incubation was done at 37^{0} C for 24 hours. Each plate of the medium was observed for colony growth. The values obtained in the plate with lowest concentration of the extracts without colony growth was recorded as the MBC/MFC (Garba *et al.*, 2019).

RESULTS AND DISCUSSION

The phytochemical analysis result as presented in Table 1, indicates the presence of pharmacologically useful classes of compounds such as, saponins, tannins, alkaloids, flavonoids, phenols, steroids, glycosides but they were however spread across the extracts for instance saponins, tannins, alkaloids phenols and glycosides were absence in methanol extract but all presence in petroleum ether. These secondary metabolites have been shown to have therapeutic activities in plants and function in a synergistic or antagonistic fashion for the treatment of diseases (Trease and Evans, 1989). Alkaloids are well known for their wide pharmacological activities ranging from anti-bacterial and antifungal (Trease and Evans 1989). The secondary metabolites detected in this study are known for their importance in industrial and medicinal sciences. Plant phenolics especially flavonoids are currently of growing interest owning to their supposed properties in promoting health (antioxidants). Flavonoids have been demonstrated to have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anticarcinogenic activity (Rauha *et al.*, 2000). Tannins are reported to possess physiological astringent and haemostatic properties, which helps in hastening wound healing and ameliorating inflamed mucus membrane and also inhibit the growth of microorganisms (Tyler *et al*., 1988).

Antimicrobial sensitivity test of *Cassia obtusifolia* leaf extracts as shown in Table 2 showed concentration dependent activity of extracts against the test organisms. The result of zone of inhibition of dichloromethane at 4000µg/ml shows zone of inhibition range from 11mm to 19mm, at 2000µg/ml zone of inhibition ranges from 10mm to 16mm, at 1000µg/ml zone of inhibition ranges from 8 to 14mm and at 500µg/ml zone of inhibition ranges from 0 to 12 mm. The result of zone of inhibition (ZI) of petroleum ether at 4000µg/ml showed inhibition range from 11mm to 13mm, at 2000µg/ml 8mm to 11mm, at 1000µg/ml 7mm to 10mm, and at 500µg/ml concentration showed 0mm to 9mm respectively. The ethyl acetate gave the highest activity with 20mm zone of inhibition against *S.aureus* at a concentration of 4000µg/ml, at 2000µg/ml inhibition ranged from 10 to 18mm, at 1000µg/ml also range from 8 to 16mm and at the lowest dosage shows 7 to 14mm zone of inhibition. The result of zone of inhibition of methanol extract at the highest dosage showed the inhibition range from 9 to 17 mm, at 2000µg/ml showed the ranges 8 to 15mm, at 1000µg/ml range 0 to 13mm and at the lowest concentration of 500µg/ml sowed 0 to 12mm respectively. But standard antibiotic (ciprofloxacin) used as positive control range from 25mm to 34mm and nystatin 22mm to 25mm. In this screening the ethyl acetate demonstrated more activity with the zone of inhibition of 20mm against *Staphylococcus aureus* and 18mm against Escherichia coli and zone of inhibition of 18mm of 2000mg/ml was also recorded against *Staphylococcus aureus.* The least MIC(1000 µg/ml) was recorded for the ethylacetate extract against *Staphylococcus aureus.* The antimicrobial activities demonstrated by the extract may be related to the presence of phytochemicals.

Table 1: Phytochemical screening of *C.obtusifolia* leaf extract

Metabolites	Methanol	Petroleum ether	Dichloromethane	Ethyl acetate
Saponin	-	+	-	+
Tannin	-	+	-	-
Steroids	+	+	+	+
Flavanoid	+	+	+	+
Triterpenoid	+	+	+	+
Alkaloid	-	+	+	+
Phenol	-	+	-	-
Glycoside	-	+	-	-

Table 2. Antimicrobial activity of *Cassia obtusifolia* leaf extracts against clinical bacterial and fungal isolates showing diameter of inhibition (mm)

Pathogens	Pet ether (µg/ml)				Dichloromethane (µg/ml)				Ethylacetate (µg/ml)				Methanol(µg/ml)				CPX	NST
	4000	2000	1000	500	4000	2000	1000	500	4000	2000	1000	500	4000	2000	1000	500		
S. aureus	13	11	10	9	11	10	9	7	20	18	16	14	13	11	9	7	34	
E. coli	11	9	7	0	19	16	14	12	18	15	12	10	17	15	13	12	27	
K.pneumonia	0	0	0	0	15	12	10	9	0	0	0	0	10	9	7	0	33	
P. mirabilis	12	10	8	7	16	14	11	10	13	11	10	8	11	9	7	0	30	
S.dysenteriae	11	10	8	0	14	11	10	8	18	15	13	11	13	10	8	7	25	
S. pyogenes	0	0	0	0	13	11	10	8	13	10	9	0	10	8	0	0	28	
S.typhi	10	9	7	0	18	15	12	11	16	13	11	9	18	8	0	0	34	
P.mirabilis	12	10	8	7	16	14	11	10	13	11	10	8	0	0	0	0	35	
P. aeruginosa	12	10	8	0	13	10	9	7	15	12	10	8	11	9	7	0	27	
Aspergillus niger	13	10	9	8	14	11	9	8	12	10	9	8	9	8	0	0	0	22
Aspergillus flavus	11	9	7	0	12	10	8	0	13	10	8	7	12	10	8	0	0	25

Key: CPX= ciprofloxacin, NST= nystatin

Table 3.Minimum Inhibitory Concentrations (MIC) and Minimum Bacterialcidal (MBC) Of Plant Extract

Isolates	**DCM (µg/ml)**		**PE (µg/ml)**		**EA (µg/ml)**		**MEOH (µg/ml)**	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	4000	++	4000	++	1000	++	4000	++
Klebsiella pneumoniae	1000	++	-	-	-	-	-	-
Streptococcus pyogenes	1000	++	-	-	-	-	-	-
Proteus mirabilis	2000	++	2000	++	++	++	-	-
Salmonella typhi	4000	++	-	-	1000	++	-	-
Shigella dysenteriae	1000	++	-	-	2000	++	1000	++
Pseudomonas aeruginosa	2000	++	-	-	1000	++	-	-

Key: ++ MBC greater than 4000µg/ml, - = no activity, ME=methanol extract, PE=petroleum ether extract, DCM=dichloromethane extract, EA=ethyl acetate extract

CONCLUSION

The result of this study indicated that the leaf of *Cassia obtusifolia* contain some major bioactive secondary metabolites that inhibits the growth of microorganism hence can be a very effective alternative source of antibiotics. The current findings lend credence to the traditional use of this plant as medicines for treating several infectious diseases. However it is imperative that more works also be done on its toxicity studies in order to determine its safety level.

REFERENCES

Akroum, S, Satta, D and Lalauoui, K. (2017). Antibacterial, antioxidant, cytotoxic activities and phytochemical screening of honey and ginger. *European Journal of Scientific Resarch*, 31 (2): 289-295.

Banjo N.O., Abikoye E.T. and Kukoye A.O. (2004). Comparison of three nutrient supplements used as additives to saw dust during the cultivation of Oyster mushroom (*Pleurotus pulmonaris*). *Nigerian Journal of Microbiology*, 18: 335-336.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C.and Turck, M. (19660. Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology*, 45: 493-496.

Cheesbrough, M. (2000): District Laboratory Practice in tropical countries, published by press syndicate of the university of Cambridge, the Edinburgh Building, Cambridge United Kingdom, New York. Pp.194-201.

Clinical and Laboratory Standard Institute (CLSI) (2015). *Performance Standards for Antimicrobial Susceptibility Testing. 25th International Supplement;* CLSI Document M100-S25; Clinical and Laboratory Standard Institute (CLSI): Wayne, PA, USA.

Fallah-Hoseini H, Fakhrzadeh H, Larijani B, Shikhsamani. (2006).A Review of anti-diabetic medicinal plant used in traditional medicine*. J Med Plant*, **5**:1–8.

Falade, O.S., Dare, A.F., Bello, M.O., Osuntogun, B.O. and Adewusi, S.R.A. (2004). Varietal changes in proximate composition and the effect of processing on the ascorbic acid content of some Nigerian vegetables. *J. Food Technol*, 2: 103-108.

Garba , l., Lawan, H.S., Yusuf, I., Abdullahi, M.M. and Puma, H.U. (2019). Phytochemical screening and invitro antibacteriostatic effect of syzigium aromaticum (clove). *Journal of Biochemistry, microbiology and Biotechnology*, 7 (1): 5-9.

Harborne JB, (1973). Phytochemical Methods.Chapman and Hall Ltd, London, UK. (1st eds.), 49-188.

Rauha, J. P., Remes S., Herinonen, W., Hopia, M., Kgjala, T., Pitinlaja, K, Vuorela, H. and Vuorela, P. (2000). Antimicrobial effects of finished plant extract containing flavanoids and other phenolic compounds. *Int.J. Food Microbiol*. 56: 3-12.

Sofowora, A, (1993). Medicinal plants and Traditional Medicine in Africa. Spectrum books Ibadan, 150.

Trease G.E. and Evans, W.C. (2005). Trease and Evans Pharmacognosy. (14th ed). WB Saunders Company limited, London. 357-358.

Trease, G.E and Evans, W.C (1989). Pharmacology 13th edition W.B Saunders Company ltd. London, . 263-266.

Tyler, V. E., Brady, L. R., Roberts, J. E. (1988). Pharmacolog. Lea and Ferbiger, Philadelphia. 85-90.

Vollekova, A., Kostalova, D., and Sochorova, R. (2001). Isoquinoline alkaloids from *Mahonia aquifolium* stem bark are active against *Melssezia* species. *Folia Microbiology,* 46:107-111.

WHO (2013). WHO Traditional Medicine Strategy. *Alternative and Integrative Medicine*, *1*(1), 1–78.

Fallah-Hoseini H, Fakhrzadeh H, Larijani B, Shikhsamani.(2006).A Review of anti-diabetic medicinal plant used in traditional medicine*. J. Med. Plant*; 5: 1-8.