

In-vitro antimicrobial activity and phytochemical screening of *Cassia obtusifolia*

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Accepted 18 August, 2017

ABSTRACT

Cassia obtusifolia is an annual or perennial plant which is used in several traditional medicines to cure various diseases. *C. obtusifolia* is a spiny herb that grows all over in India in shade as well as under open condition. Generally found up to an altitude of 1,000 m in Himalaya and wild throughout the plains on waste lands or in the coastal areas. It is also found in deltaic region of western, eastern and southern India. The crude was extract by ethanol using a maceration method. The ethanolic extract of *C. obtusifolia* seeds were tested against three standard bacterial species: one Gram-positive bacteria viz., *Staphylococcus aureus* (ATCC 25923), two Gram-negative bacterial strains *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), using the disc diffusion method and phytochemical screening. The study was aimed to investigate antimicrobial activity and phytochemical screening of *C. obtusifolia* ethanolic crude extract. The results of phytochemical screening showed the presence of Flavonoids, Saponins, Alkaloid, Tannins, Phenols, Triterpene, Glycosides, Resins, Steroids and Carboxylic acid. The extract of *C. obtusifolia* dissolved in methanol (1:10) showed low activity (17 and zero mm) against Gram negative bacteria (*P. aeruginosa* and *E. coli*) and (zero mm) against (*S. aureus*). This study give rise to antioxidant property of studied plant, and showed interesting correlation with the phytochemical constituents and biological activities.

Keywords: *Cassia obtusifolia*, antimicrobial activity, phytochemical, sickle pod.

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INTRODUCTION

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. At least 12,000 compounds have been isolated from plants; a number estimated to be less than 10% of the total. Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs (Tapsell et al., 2006).

Much more ancient, albeit less conclusive, evidence suggests that humans might have employed the pharmacological properties of plants much earlier. At the famous burial site in the Shanidar Cave in the northern part of Iraq, a Neanderthal (*Homo neanderthalensis*) was

laid to rest with bunches of flowers about 60,000 years ago (Solecki, 1975). Of the eight plants identified in the grave from preserved pollen, seven are considered medicinal plants today. There is of course no way of knowing with certainty whether they were placed in the grave because of their medicinal properties, to serve the dead man on his final journey, or whether they simply were used for decorative purposes. The more recent discovery of the 'Iceman' on the Italian-Austrian border in the Alps provides intriguing evidence of early use of medicinal fungi in Europe. This hunter, who had been lying well preserved in the ice for about 5300 years, was found to be in possession of a fungus, the birch polypore (*Piptoporus betulinus*), which is known to have purgative and antibiotic properties, and which he might well have been using to treat the whipworm infestation of his intestines (Heinrich et al., 2004).

Cassia obtusifolia (Chinese senna, American sicklepod or sicklepod) is a legume in the genus *Senna*, sometimes separated in the monotypic genus *Diallobus* (Botanical Society of Britain and Ireland, 2014). It grows wild in North, Central, and South America, Asia, Africa, and Oceania, and is considered a particularly serious weed in many places. It has a long-standing history of confusion with *Senna tora* and that taxon in many sources actually refers to the present species.

The green leaves of the plant are fermented to produce a high-protein food product called "kawal" which is eaten by many people in Sudan as a meat substitute (Dirar, 1984). Its leaves, seeds, and root are also used in folk medicine, primarily in Asia. It is believed to possess a laxative effect, as well as to be beneficial for the eyes. As a folk remedy, the seeds are often roasted, then boiled in water to produce a tea. The plant's seeds are a commercial source of cassia gum, a food additive usually used as a thickener and named for the Chinese *Senna's* former placement in the genus *Cassia*. Roasted and ground, the seeds have also been used as a substitute for coffee. In traditional Korean medicine, they are called *gyeolmyeongja* and usually prepared as tea. They are also used in *Kampō* (traditional Chinese medicine in Japan), where they are called *ketsumei-shi* or by their Chinese name *jué míng zǐ*.

C. obtusifolia is regarded as 'Edible weeds of Agriculture or Famine food'. Its infusion is given against the white discharge. In Mali, *C. occidentalis* is used as ingredient in a malarial formulation based on a traditional recipe comprising leaf of *C. obtusifolia* leaves of *Lippia chevalieri* and flower heads of *Spilanthes oleraces* (Willcox et al., 2012). Decoction of *C. obtusifolia* roots with black pepper is useful in filarial (Arvind and Shamshun, 2007). In the Malyagiri hills, a decoction is made from 15 leaves each of *C. occidentalis*, *Glycosmis pentaphylla* and *Vitex negundo* and used for bathing the new born baby to make the baby immune to skin diseases (Yadav et al., 2010). According to 'Bhavaprakasa', Kasamarda (*C. occidentalis*) is used in constipation, and is stated that leaves, roots and seeds are useful as purgative (Warrier and Nambiar, 1994). In folklore medicine, seed powder (half a tea spoon) is used to cure fever while two table spoons of leaf juice mixed with honey cures cough. For intestinal gas half a cup of leaf decoction is taken twice daily and paste of leaf is applied for skin diseases. The seeds of *C. obtusifolia* exert their toxic effect on the skeletal muscles, kidney and liver. The green and dry leaves and the stem also contain toxin (Osman et al., 1972) found, through rat feeding experiments, that kawal at concentration of 2.5% in the diet did not cause any significant changes in body weight as compared with a control group. However, rats fed on diet containing 10 or 50% kawal showed a highly significant weight loss. The use of toxic plants as food after fermentation or heat treatment is known in Africa and other parts of the world (Dirar, 1993). The most important microorganisms of kawal fermentation are

Bacillus subtilis and *Propionibacteria* spp., followed by *Lactobacillus plantarum* and *Staphylococcus sciuri* sub *lentis* (Dirar, 1993) and moulds including *Rizopus* species (Dirar, 1985). Phytate-degrading enzymes have been detected in various bacterial genera, such as *Bacillus* (Kerovuo et al., 2000) and *Pseudomonas* (Richardson and Hadobas, 1997). These bacterial genera have been isolated from fermenting fluted pumpkin seeds (Barber et al., 1989). Reduction in polyphenols may be due to the activation of polyphenoloxidase during fermentation process (Dhankher and Chauhan, 1987). Therefore, the study was aimed to investigate antimicrobial activity and phytochemical screening of *C. obtusifolia* (seeds).

MATERIALS AND METHODS

Plant materials

The plant of Sickle pod (*C. obtusifolia*) was collected from Khartoum central Sudan during September to October 2016, and the plant was kindly identified and authenticated by Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI) in Khartoum, Sudan. Seeds were air-dried, under the shade, pulverized and stored prior to extraction. Shade with good ventilation and then ground finely in a mill and kept in the herbarium until crude extract preparation (Figure 1).

Extraction of crude sample

Fresh plant samples were dried in shades for 7 days, powdered and then used for extraction. Extraction was carried out according to the method described by Harbone (1984). The shade-dried samples were soaked in 95% ethanol at a ratio of (1:10) at room temperature for 7 days, filtered left to dry at room temperature. This process was repeated till the solvent returned to colourless. The weight of the solid residues was recorded and taken as yield of crude extracts. Yield percentage was calculated as follows:

$$\text{Yield \%} = (\text{weigh of extract/weigh of sample}) \times 100$$

Qualitative phytochemical evaluation

Phytochemical screening was conducted to determine the presence of natural products in the extracts samples using standard methods (Trease and Evans, 1989; Odebiyi and Sofowora, 1978).

Phenols (ferric chloride test)

To 1 ml of extract, 2 ml of distilled water were added



Figure 1. *Cassia obtusifolia* (seeds).

followed by few drops of 10% ferric chloride (FeCl_3). Appearance of blue or green colour indicates the presence of phenols.

Flavonoids

Three different tests were used for the identification of flavonoids.

Potassium hydroxide (KOH) test

About 1 ml of extracts was treated with few drops of 10% potassium hydroxide solution. Formation of intense yellow colour indicates the presence of flavonoids.

Alkaline test

About 0.5 ml of extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Lead acetate test

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Tannins (ferric chloride test)

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 blue, greenish black colour change.

Alkaloids

Two different tests were used for the identification of alkaloids.

Dragendroff's test

Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

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 is observed for the formation of reddish brown precipitate.

Triterpenes and steroids (Salkowski test)

Salkowski test was used to identify steroid and terpenoid. To 0.5 ml of each extract, 2 ml of chloroform was added and then 3 ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration at the interface indicates the presence of terpenoids and steroids.

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.

Test for glycosides

Five ml each of various extract were hydrolysed

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.

Test for resins

One ml of various solvent extract were treated with few drops of acetic anhydride solution followed by one ml of conc. H₂SO₄. Resins give colouration ranging from orange to yellow.

Test for carboxylic acid

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

Test microorganisms

The ethanolic crude extract of *C. obtusifolia* was tested against three standard bacteria species: one Gram-positive bacteria viz., *S. aureus* (ATCC 25923), two Gram-negative bacterial strains *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853), using the disc diffusion method. The standard bacterial strains used in the study were obtained from the Department of Microbiology, Faculty of Medical Laboratory, International University of Africa, Khartoum, Sudan. The bacterial cultures were maintained on nutrient agar and incubated at 37°C for 18 h and then used for the antimicrobial test.

Testing of antibacterial and anti-fungi susceptibility

Disc diffusion method

The paper disc diffusion method was used to screen the antibacterial activity of plant extract and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 10⁸ cfu/ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 min. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of plant extract. The inoculated plates were incubated at 37°C for 24 h in the inverted position. The

diameters (mm) of the inhibition zones were measured.

Statistical analysis

All data were presented as means ± S.D. Statistical analysis for all the assays results were done using Microsoft Excel program (2010).

RESULTS AND DISCUSSION

The ethanolic extract of *C. obtusifolia* seeds family (Fabaceae) was screened for antimicrobial activity against three bacterial species: one Gram-positive bacteria viz., *S. aureus* (ATCC 25923), two Gram-negative bacterial strains *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853), using the disc diffusion method and phytochemical screening.

Table 1 indicates the presence of pharmacologically useful classes of compounds (Flavonoids, Saponins, Alkaloid, Tannins, Phenols, Triterpene, Phytosterol, carboxylic acid) tested for. 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, 1996).

The various phytochemical compounds detected are known to have beneficial importance in industrial and medicinal sciences. Plant phenolic compounds especially flavonoids are currently of growing interest owing to their supposed properties in promoting health (anti-oxidants) (Rauha et al., 2000); flavonoids have been demonstrated to have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity. The broad therapeutic effects of flavonoids can be largely attributed to their antioxidant properties. In addition to an antioxidant effect, flavonoid compounds may exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macrophages (Percival, 1998).

Tannins are reported to possess physiological astringent and haemostatic properties, which hasten wound healing and ameliorate inflamed mucus membrane and also inhibit the growth of microorganisms by precipitating microbial proteins and making nutritional proteins unavailable for them; they form irreversible complexes with proline rich proteins, resulting in the inhibition of the cell protein synthesis. They have important roles such as stable and potent anti-oxidants (Tyler et al., 1988; Awosika, 1991; Ogunleye and Ibitoye, 2003). They act as binders and for treatment of diarrhea and dysentery (Dharmananda, 2003). Tannin also reported to exhibit antiviral, antibacterial, anti-tumor activities. It was also reported that certain tannin are able to inhibit HIV replication selectivity and is also used as diuretic (Heslem, 1989). Tannin also reported to exhibit antiviral, antibacterial, anti-tumor activities. It was also

Table 1. Phytochemical screening of *Cassia obtusifolia* seed extract.

S. No.	Phytochemical constituent	Type of test	Result
1	Phenols	FeCl ₃	+v
2	Flavonoid	KOH	+v
		Alkaline test	+v
		Lead acetate	+v
3	Tannine	FeCl ₃	+v
4	Alkaloid	Dragendorff's	-v
		Wagner's	-v
5	Steroids	Salkowski	+v
6	Terpenoid	Salkowski	+v
7	Saponins	Forth	+v
8	Glycoside		+v
9	Resine	acetic anhydride	-v
10	Carboxylic acid	sodium bicarbonate	+v

Table 2. The antimicrobial activity ethanolic extract of *Cassia obtusifolia* and reference antibiotics against the standard bacteria.

Standard microorganisms	Concentration (mg/ml)				Gentamicin 30 µg/ml
	Mean diameter of growth inhibition zone (mm)				
	100	50	25	12.5	
<i>Escherichia coli</i>	-	-	-	-	29
<i>Staphyococcus aureus</i>	-	-	-	-	30
<i>Pseudomonas aeruginosa</i>	17	15	-	-	25

reported that certain tannin are able to inhibit HIV replication selectivity and is also used as diuretic (Heslem, 1989). Plant tannin has been recognized for their pharmacological properties and is known to make trees and shrubs a difficult meal for many caterpillars (Aiyelaagbe and Osamudiamen, 2009). Plant steroids are known to be important for their cardiotoxic, insecticidal and anti-microbial properties. They are also used in nutrition, herbal medicine, cosmetics and they are routinely used in medicine because of their profound biological activities (Denwick, 2002). Saponins have expectorant action which is very useful in the management of upper respiratory tract inflammation; saponins present in plants are cardiotoxic in nature and are reported to have anti-diabetic and anti-fungal properties (Finar, 1989; Trease and Evans, 1989; Kamel, 1991).

The extract of *C. obtusifolia* dissolved in methanol (1:10) showed Low activity (17 and zero mm) against Gram negative bacteria (*P. aeruginosa* and *E. coli*) and (zero mm) against (*S. aureus*) (Table 2). This result showed that the extract tested inhibited the growth of all

microorganisms though the sensitivities of microorganisms varied. This result was similar to that reported by Seidler-Lozykowska et al. (2013), who found that the plant extract showed low activity against *S. aureus* and *E. coli*.

C. obtusifolia ethanolic extract inhibited the growth of *P. aeruginosa* bacteria (Shan et al., 2005) Thus, according to our investigation *C. obtusifolia* cannot be used as a potent as antimicrobial agent for human pathogens.

CONCLUSION

Thus, from the present study the plant seeds extracts of *C. obtusifolia* showed an abundant production of phytochemicals compounds with biological activities. They can be used in the pharmaceutical industries for producing a potent drug against pediculosis and increasing male sex vigour. The studies result of the above one plant gives a basis of its use in traditional medicine to manage ailments and disorders. It also contains some biologically active constituent's worthy of

further investigations.

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Citation: Ali M. M., Ali S. S., Abdalaziz M. N. (2017). *In-vitro* antimicrobial activity and phytochemical screening of *Cassia obtusifolia*. *Adv Sci Technol Res*, 3(2): 15-20.
