JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2012. Vol. **6**(3), p. 1433-1436

Evaluation of the Role of *Cassia occidentalis* Extracts as Antimicrobial Agents

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(Received: 30 April 2012; accepted: 09 June 2012)

Flower and leaf extracts of *C.occidentalis* have shown antimicrobial activity. Extracts were more effective to *Streptococcus* sp than that of *Aspergillus* sp. Ethyl acetate flower extract of *C.occidentalis* showed more effectiveness than other extracts. Qualitative analysis of flower extracts revealed the presence of phenolics, steroid, flavonoid, terpenoid, glycoside. Diethylether extract of *C. occidentalis* contained more phenolic compounds than other extracts while methanolic extracts contained more flavonoid compounds than other fractions.

Key words: *Cassia occidentalis,* Phytochemicals, Antimicrobial effect.

Traditional medicine (also known as indigenous or folk medicine) comprises medical knowledge that develops over generation within various societies before the era of modern medicine. Traditional medicine include herbal, ayurveda, acupuncture and other medical knowledge and practices all over the globe. WHO defines traditional medicine as "the health practices, approaches, knowledge and beliefs incorporating plant , animal and mineral based medicines ,spiritual therapies, manual techniques and exercises applied in or in single or in combination to treat, diagnose and prevent illness or maintain well being".

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More than 2000 plant species have already been recorded to have insecticidal properties (Balandrin, 1985 and Rawls, 1986) . Natural insecticides like pyrethrum from *Chrysanthemum* sp, rotenone from *Derris*, among others, have been widely used for insect pest and vector control (Balandrin, 1985). About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants extracts should be evaluated to study their properties, safety and efficiency. Here we have selected *Cassia occidentalis* for our study to evaluate its antimicrobial role. The general name of *Cassia* in English is Negro coffee and in Hindi it is Kasundi. The *Cassia* plant is generally found in tropical and temparate region, mainly in India and Southern Asia. The height is 2-4 feet. Flowers are yellow in colour. Flowers appear in autumn and fruits in winter. The plant is aromatic in nature. *C.occidentalis* known to have good effect on skin related ailment, analgesic, normalizes digestion. It has no toxic effect on human body. The whole plant is useful.

MATERIALS AND METHODS

Collection of Plants

The plant *C.occidentalis* was collected from Golapbag area of Burdwan from West Bengal.

Preparation of plant extracts

Different parts like, root, stem, leaves, flowers of the plant *C. occidentalis* were taken. The plant parts were crushed in different solvents like water, methanol, benzene, chloroform & ethyl acetate using mortar and pestle and in this way extracts were prepared. Firstly crude extracts were used, later the extracts were centrifuged and the supernatant were taken for further use. At the same time overnight saturated extracts with respective solvents were also used.

Preparation of sample for identification of active ingredients

The dried residue of chloroform : methanol extract was eluted with absolute alcohol and subjected to TLC on silica gel G (thickness 0.5 mm), which had been prepared using a with benzene : ethyl acetate (1:1) as the mobile phase. The TLC plates were sprayed with different spraying reagents for identification of biochemicals (Table 1). The phytochemical analysis was carried out following the standard method (Harborne, 1984). Spray reagents Acetic anhydride-sulphuric acid (Liebermann-Burchard reagent) (5 ml acetic acid anhydride mixed with 5 ml concentrated sulphuric acid and 50 ml absolute ethanol was added after cooling), Antimony chloride reagent (10% solution of Antimony chloride in chloroform), Ceric sulphate-sulphuric acid reagent(saturated solution of Ceric sulphate in 65% sulphuric acid), Dragendorff's reagent (0.85 g basic Bismuth nitrate was dissolved in a mixture of 10 ml acetic acid and 40 ml water, with it equal volume of a mixture of 8 g potassium iodide dissolved in 20 ml water was added), Folin Ciocalteu reagent, Formaldehyde-phasphoric acid reagent (0.3 g para formaldehyde dissolved in 100 ml 80% phosphoric acid) and Ninhydrin silver nitrate reagent (0.3 g ninhydrin dissolved in 100 ml n-butanol and 3ml acetic acid was added) were used to detect the biochemical constituents.

Agar -cup Assay

Microbial susceptibility assays were done following NCCLS (NCCLS,2003). Agar cup method was followed. Culture of *Staphylococcus* sp., *Bacillus* sp., *Pseudomonas* sp., *E.coli* DH5α, *Streptococcus* sp., *V.cholerae*, *Rhizobium* sp., *Agrobacterium* sp. were spread out on the Muller Hinton agar plate. Wells were made on the plate with a cork-borer (of diameter 1.2cm) to which water extracts were added in a specific volume. All the plates were then incubated for 24 hours at 37ºC. Rests of the extracts in other solvents were kept for further use.

Phenol estimation

Phenol estimation was done by Folin-Ciocalteau (FC reagent) method to detect the

Table 1. Inhibition zone (cm) against different extracts of *C.occidentalis*

Flower(F)/leaf(L) extracts	Zone of inhibition (cm)	
	Streptococcus sp	*Aspergillus* sp
Chloroform extract(F)	2	1.7
Ethyl acetate extract(F)	3	1.6
Benzene extract(F)	2.5	-
Methanol extract(F)	2.1	1.7
Chloroform extract(L)	1.6	1.3
Ethyl acetate extract(L)	2.5	-
Benzene extract(L)	-	-
Methanol extract(L)	1.7	1.5
1st fraction(ethyl acetate) after column chromatography	2.8	2.25
2nd fraction(ethyl acetate) after column chromatography	5.3	7

Table 2. Detection of biochemicals present or absent in flower extracts of *C.occidentalis on* TLC glass plate following treatment of different spraying reagents

Name of the solvent	Name of the spraying reagent	Result	Conclusion
Chloroform	Acetic anhydride-sulphuric acid (Liebermann-Burchard reagent)/ Vanillin – phosphoric acid	Positive	Presence of steroids(++)
Ethyl acetate – Benzene (1:1)	Folin Ciocalteu reagent	Positive	Presence of phenolics
Chloroform-Acetic acid-Water (90:45:6)	Saturated alcoholic sodium acetate	Positive	Presence of flavonoids(++)
Chloroform on Silica gel plate treated with Silver nitrate	Antimony chloride in chloroform	Positive	Presence of tarpenoids(++)
Methanol-Concentrated Ammonium hydroxide (200:3)	Dragendorff's reagent	Negative	Absence of alkaloid substance
Acetone-Hexane (4:1)	Antimony Chloride in concentrated Hydrochloric acid	Negative	Absence of steroid sapogenins

Strong positive: + +, partial positive: + , negative: -

Presence of phenolic compounds in different part of the plants using Phenol (Qualigenes) as standard (Bray and Thrope, 1954). 1ml of different part of plant extracts were taken in test tubes. 2ml of distilled water was added. 0.5ml of FC reagent was added. After 3 minutes 2ml of 20% sodium carbonate was added. The tubes were kept in boiling water bath (≈ 80ºC) for 1 minute and then cooled. Absorbance was taken at 650nm against a blank.

Flavonoid estimation

Flavonoid estimation was also done to detect flavonoid compound using Aluminium chloride (10%) colorimetric assay using Catechin as standard and absorbance were taken at 510 nm against a blank. 1 ml of different part of plant extract was taken in test tubes. 4ml of distilled water was added. 0.3ml of 5% $NaNO_2$ was added. After 5 minutes 0.3ml 10%$AlCl_3$ was added. Again after 5 minutes 2ml of 1(M) NaOH was added. Volume was made upto 10ml. Absorbance was taken at 510 nm against a blank.

Column Chromatography and antimicrobial activity of fractions

For packing of the column, Silica gel (60-120 mesh, HIMEDIA) was used. Silica gel was taken into the glass column and ethyl acetate (MERCK) was added slowly to saturate the gel. Next, extracts were taken and added to the column one by one. The extracts used were leaf extract in Benzene, Ethyl acetate, Chloroform and Methanol, and flower extract in Benzene, Ethyl acetate, Chloroform and Methanol. For each extract two fractions were collected. First fraction and second fraction were collected with ethyl acetate and petroleum ether (MERCK) respectively. In this manner, totally sixteen fractions were collected from eight extracts. Fractions were further tested for Agar cup assay to check the antimicrobial activity against *Streptococcus* sp., *Vibrio cholerae*, *Staphylococcus aureus, Rhizobium* sp., *Bacillus* sp. The plates were prepared by media. The *culture* was spread over the Muller Hinton Agar media. In each plate, two wells were loaded with 750 µl plant

extracts. One plate was used as control having crude ethyl acetate in one well and crude petroleum ether in another well. The plates were kept for incubation at 37°C for 24 hrs.

RESULTS AND DISCUSSION

Although different plants of several families have been reported for insecticidal activity (Green *et al.*, 1991), but only a few botanicals have been exploited for field use. Different extracts of *C.occidentalis* have shown antimicrobial activity. *Streptococcus* sp. was found more sensitive to the flower and leaf extracts of *C.occidentalis* than that of *Aspergillus* sp. Ethyl acetate flower extract of *C.occidentalis* was found to be more effective than other extracts. (Table 1). By qualitative analysis of flower extracts were found to be positive for phenolics steroid, flavonoid, terpenoid, glycoside, but negative for phlobatannin, alkaloid,saponin, tannin and anthraquinone (Table 2). By quantitative estimation,diethylether extract of *C. occidentalis* has been shown to contain more phenolic compounds than other extracts while methanolic extracts has been shown to contain more flavonoid compounds than other fractions. Although the drug industries have produced a number of new antibiotics in the last three decades, bacterial resistance to these drugs has also been increased due to the genetic ability bacteria to transmit and acquire resistance to drugs (Cohen, 1992). A high incidence of resistant microorganisms was reported (Montelli and Levy, 1991). According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. Plant extracts and phytochemicals, having antimicrobial properties are of great significance in therapeutic treatments. A number of studies have been conducted in different countries in the last few years in order to prove such effectiveness (Almagboul *et al.*, 1985; Artizzu *et al.*, 1995; Ikram and Inamul, 1984, Izzo *et al.*, 1995). Present work revealed the importance of plant extracts of *C.occidentalis* to control bacteria which are becoming a threat to human health.

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