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Studies on the phytochemistry, antimicrobial activity and antioxidant properties of *Cassia occidentalis L.*

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ABSTRACT

Antibiotic resistance has become a global concern. Antibiotics exist in indispensable for today's pharma medical market. Despite that their usage is become increasingly restricted. The reason being attributed largely to the development of drug resistance among microorganisms. In addition to that, the phenomenon is coupled by the toxicity possessed by many of the conventionally prescribed antimicrobials, warranting a search for newer antimicrobial agents. In view of increasing resistance to existing antimicrobial agents, herbal drugs looked as very important source for treating various microbial infections. There has been worldwide move towards the use of traditional medicines due to concern over the more invasive, expensive, potentially toxic main stream practices. Numerous studies have identified compounds with in herbals that are effective antibiotics. The pathogenic microbial agents less susceptible to regular antibiotics and the rising trend in the recovery rates or resistant bacteria highlight the need for newer alternative principles. This present study is aimed to investigate phytochemical, Antioxidant and antimicrobial activities of various parts of medicinal plants.

Key Words: *Cassia occidentalis*, Phytochemical, Antibacterial and Antioxidant

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INTRODUCTION

Infectious diseases are disorders caused by pathogenic microorganisms like bacteria, viruses, fungi, protozoa and multi cellular parasites. These diseases are also called as communicable or transmissible diseases since they can be transmitted from one person to another via a vector or replicating agent. Infectious diseases account for about half of the deaths in tropical countries (Khosravi and Behzadi, 2006). Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases (Stary and Hans, 1998). Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and infectious diseases.

Cassia tora L. (Family: *Cesalpinaceae*), *Calendula officinalis* (Family: *Compositae*) and *Momordica charantia* (Family: *Cucurbitaceae*) are some of the very common Indian herbs having various medicinal properties for the treatment of different kind of disease, viz. antifungal, wound healing and antidiabetic agents respectively (Nadkarni, 1982; Brown and Dattner, 1998; Grover and Yadav, 2004; Christopher, 2005) . These herbs have been reported for their usefulness in the form of decoctions, infusions and tinctures in traditional system of medicines for treating skin diseases like psoriasis, leprosy etc (Horvath and Ferenc, 1992; Zahra *et al*., 2000; Cordova et al., 2002; Harrison and Dorothy, 2003).

MATERIALS AND METHODS

Plant material and preparation of the Extract

The plants were collected from uncultivated farmlands located at Southern parts of Tamilnadu in Thanjavur. All the plant samples were identified by the authors. The plant samples were air-dried and ground into uniform powder using a Thomas- Willey milling machine. The powder was used for extraction of bioactive compounds. The aqueous

extract of each sample was prepared by soaking 100 g of dried powdered samples in 200 ml of distilled water for 12 h. The extracts were filtered using Whatman filter paper No. 42 (125 mm).

The phytochemical study deals with the estimation of phenolics, flavonoids and carotenoids, present in the powder and aqueous extract of. *Cassia occidentalis* seeds

PHYTOCHEMICAL ESTIMATIONS

Phytochemical screening tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

I. QUANTITATIVE ANALYSIS

Extraction and Estimation of Phenolics(Price et al., 1980)

25µl of acetone and 60µl of ferric ammonium sulphate were added to plant extract and kept at room temperature for 20 minutes. To this, 60µl of potassium ferricyanide was added and the absorbance was measured at 720 nm after 20 minutes by incubation at room temperature. Quercetin was used as a standard and the total phenolics were expressed as µg/gm of extract.

Extraction of Flavonoids (Harborne, 1975)

Flavonoids are generally present as their glycosides. They are hydrolised and free flavonoids were assayed.) The extract (1ml) was mixed with 1ml of 2% methanolic $AlCl_3$ and the absorbance was measured at 430 nm. Quercetin dissolved in methanol in the range of 100-500µg was used as standard.

Extraction and estimation of total Carotenoids (Narayanaswamy & Palanisamy, 1973)

One gram of the sample was homogenized. until it was free from pigments. The filtrates were pooled and partitioned with equal quantity of peroxide free ether,. The ether phase which contains the carotenoids was evaporated and the residue was dissolved in 1ml of ethanol. and absorbance was measured at 450 nm.

$C = D \times V \times F / 2500$

II ESTIMATION OF METAL IONS IN *Cassia occidentalis*

Aqueous extract of *Cassia occidentalis* was digested according to the method of Ballentine & Buford, (1957). The cadmium, chromium, lead, mercury and nickel were estimated in the digest by atomic absorption spectroscopy (AAS). .

II. *IN-VITRO* ANTIOXIDANT STUDY: Scavenging of 1,1- Diphenyl-2-picryl hydrazyl (DPPH) radical (Yokazawa et al., 1998)

This assay is based on the measurement of the scavenging ability of antioxidant test substances towards the stable DPPH radical.

The free radical scavenging activity of the aqueous extract of was examined *Cassia occidentalis in-vitro* using DPPH radical. -HCl buffer (pH 7.4), 1ml of ethanol and 0.05ml of the *Cassia occidentalis* extract. The absorbance of the mixture was measured at 517 nm. Ascorbic acid was used as reference standard. The% of scavenging activity was calculated using the formula 100−[100/blank absorbance × sample absorbance].

Organisms used

Escherichia coli, *Pseudomonas sp.* and *Staphylococcus sp.* were isolated from clinical specimen. Isolated bacteria were identified based on morphological and biochemical characteristics. Log phase cells were used for assays.

Analysis of Bacterial Activity

The antibacterial activity of crude *Cassia occidentalis* L. leaf extract and were tested by the agar diffusion method. For the determination of antibacterial activity, the antibiotic resistant bacteria namely, *Staphylococcus sp, Escherichia coli and Pseudomonas aeruginosa* used

RESULTS AND DISCUSSION

In the present study Escherichia coli, Pseudomonas sp. and Staphylococcus spp, were isolated from clinical specimen. Isolated bacteria were identified based on morphological and biochemical characteristics. The results were presented in table -1. Phytochemical analysis of aqueous showed the presence of anthraquniones,

carbohydrates, glycosides, cardiac glycosides, amino acid, phytosterols, fixed oils and fats, phenolic compounds, tannins, flavonoids, steroids and saponins while alkaloids are absent in all of the tested extracts (Table – 2&3). Herbal drugs contain unique constituents which differs from one herb to another, hence the type and extent of their medicinal property also differs. The free radical scavenging activity of the aqueous extract of *Cassia occidentalis* was studied using DPPH radical (Table – 4)(Le G A. 1989; Evans WC. 1996) Aqueous extract of *Cassia occidentalis* was tested for the presence of metal ions.(Table5)Solubility of each constituent in an herb is very specific to different solvents used in the extraction process. Hence, chemical nature as well as the pharmacological activity of herbal extracts obtained using same herb with different solvents will be different. (Kirtikar and Basu 1999)

The antibiotic sensitivity of bacteria such as Escherichia coli, Pseudomonas spp and Staphylococcus spp, against various antibiotics was analyzed. The organisms showed resistance to antibiotics such as Ampicillin, Chloramphenicol, Streptomycin and Tetracycline (Table - 6). Aqueous extract was found to have maximum zone of inhibition against Escherichia coli, Pseudomonas spp, and Staphylococcus spp, .). Literature also reveals the use of aqueous paste of seeds of *Cassia occdentalis* , Momordica charantia seed juice (prepared in water) and Calendula flower water decoctions in various skin conditions associated with bacterial infections. (Nadkarni, 1982,)

Table-1: Morphological and biochemical characteristics of Isolated bacteria

S. No.	Morphological and Biochemical characteristics	Results		
		Escherichia coli	*Pseudomonas aeroginosa*	*Staphylococcus sp*
1.	Motility	+	+	-
2.	Gram staining	-/Rod	-/Rod	-/Coccus
3.	Indole test	+	-	-
4.	Methyl red test	+	-	-
5.	Voges Preskaur test	-	-	-
6.	Citrate test	-	+	-
7.	Triple sugar iron test	A/G	A	A
8.	Urease test	+	+	-
9.	Catalase test	+	+	-
	Oxidase	-	+	+
	Carbohydrate Fermentation test (S/G/L)	+/+/+	-/-/-	+/+/-

'+' - Positive; '-' - Negative; 'A' – Acid; 'G'- Gas

Table – 2: Phytochemical analysis qualitative analysis

S. No.	Phyto constituents	Cassia occidentalis aqueous extracts	
		Leaf	Seed
1	Alkaloids	-	+
	Tannins	+	+
2	Saponins	+	+
	Carbohydrate	+	+
3	Glycoside	-	+
	Phytosterols	+	+
4	Oils and Fats	+	+
	Phenol	+	+
5	Flavonoids	-	+
	Protein and amino acid	+	+

+' - Positive; '-' - Negative;

Table – 3: Phytochemical analysis quantitative analysis

Name of the component	Concentration of the component
Flavonoids	3.24µg/g
Carotenoids	2.9µg/g
Phenolics	6.7µg/

Table – 4: *Invitro* DPPH radical scavenging antioxidant activity

Concentration (mg/g)	Test O.D	Control(standard OD)	Percentage of inhibition
10	0.170	0.200	15
25	0.280	0.200	40
50	0.320	0.200	60
75	0.381	0.200	90.5
100	0.392	0.200	96

Table-5: Heavy Metal Analysis

Serial number	Parameter analysed	Result(µg/100g)
1	Arsenic	ND
2	Cadmium	0.028
3	Mercury	ND
4	Nickel	0.301
5	Lead	0.053

Table-6: Antibiotic Sensitivity of isolated bacteria

S. No.	Isolated Bacteria	Zone of inhibition (mm)				
		Standard Antibiotics				plant crude extracts
		Ampicillin	Chloramphenicol	Streptomycin	Tetracycline	
1	*Escherichia coli*	Resistant	8	10	Resistant	15
2	*Pseudomonas Spp*	Resistant	11	8	12	12
3	*Salmonella spp*	Resistant	12	Resistant	8	16

CONCLUSION

Finally concluded *Cassia occidentalis* aqueous plant extract can be used as antimicrobial agent antioxidant and also used as herbal medicine for curing number of disease in the form of pellets of paste. . Plant extract is being eco friendly and very cost effective; the presented method can be economic and effective alternative t h e r a p h y

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