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Original Research Paper

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PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF AND *CASSIA AURICULATA*

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ABSTRACT Phytochemical studies together with pharmacological tests have managed to integrate bioactive agents as an alternative solution to reduce or regulate the problems to several diseases. The presence of various phytochemicals and pharmacologically important compounds were investigated from Cassia auriculata for their medicinal use. The plant extracts were prepared in different solvents like methanol, hexane, chloroform and ethyl acetate. Qualitative analysis of phytochemicals were assessed, The results of phytochemical analysis suggests many bioactive and phytosteroids were present. The anti-microbial, anti-oxidant activity was determined. Anti-bacterial activity against Bacillus, E. coli, Staphylococcus and Pseudomonas was investigated. The extracts showed highest antibacterial and antioxidant activity. From the results it is clear that the extracts has pharmacological applications. This is the first report of antimicrobial, antioxidant activities. Further studies are needed to exploit the actual mechanism and active compounds of these plants.

KEYWORDS : *Cassia auriculata,* Phytochemicals, Antioxidants

INTRODUCTION

Cassia auriculata commonly known as Tanner's Cassia is an important medicinal shrub in Asia. C. auriculata (family; Caesalpiniaceae), profoundly used in tonic, astringent and as a remedy for diabetes, conjunctivitis and ophthalmia. The flowers were widely used in Ayurveda tradition as avaraipanchanga chooranam and the main constituent of Kalpa herbal tea used to treat urinary infections, nocturnal emissions and throat irritation etc., The root powder acts as a coagulant, prevents diarrhoea, dysentery, skin ailments and used for treating indigestion. The plant contain phytochemical constituents such as alkaloids phenols, glycosides, flavonoides, tannins, saponins, proteins, carbohydrates andanthraquinones etc., The bioactive compounds obtained from the plant has been widely used in traditional system of medicinal as a cure for rheumatism and other pharmacological importance. The plant has been reported to possess antipyretic hepatoprotective, antidiabetic, antiperoxidative and antihyperglycemic and microbicidal activity. Tanner's cassia is extensively cultivated in area which is dry and warm tropical regions. Ecologically cassia tolerates a wide range of climate and temperature, though it tends towards loving warmth. Cassia auriculata thrives on dry stony hills and on black soils, along road side, in degraded forest, and waste land. It is the source of yellow coloured dye obtained from its flowers and seeds (Chandramouli, 1995). In India it is found in western region like that of Rajasthan, Maharashtra, Gujarat and in southern parts like Tamilnadu, Andra Pradesh etc., Seeds are used in ophthalmia and desentry (Kirtikar and Basu, 1988). Dried flowers and leaves of C. auriculata are being used for medicinal treatment (Sawhney et al., 1978). C.auriculata have been shown to posses antibacterial, antifungal (Abo et al., 2000; Nebedum et al., 2009), antiprotozoal (Obdozie et al., 2004; Moo- Puc et al., 2007), antidiabetic activities (Jalalpure et al., 2004) and larvicidal activity against mosquito species (Yang et al., 2003; Georges et al, 2008). C.auriculata medicinal properties are due mainly to the content of hydroxyanthra quinone derivative (Yang et al., 2003). Although cassia species have been used widely to treat disease; they have shown marked toxicity to man and livestock resulting fatalities following overdoses of remedies involving the plants.

MATERIALS AND METHODS

C.auriculata leaves were air dried and pulverized into powder. About 20 gm of the powdered sample was taken in 200 ml of different solvent (methanol, Hexane, chloroform and Ethyl acetate) were added and extracted in a Soxhlet apparatus serially at 62-77°C. The filtrate was evaporated at room temperature and stored at - 20°C for further use. The active phytochemical were analysed as per standard detection methods.

ANTI-OXIDANT ACTIVITY

The DPPH activity was determined by the method of (Brand Williams et al 1990). A 200gm sample in 100ml volumetric flask was taken and made up the volume with methanol. Then 5ml sample was diluted to 50ml with same solvent. Then from this solution 50,100,200,300 and 400ml sample was taken for analysis in a set of clean and dry test tubes containing methanol (Total volume of methanol + sample should be 1ml) and 2ml of 0.1mMDPPH solution. Mixed thoroughly and kept in dark for 1hr. Similarly control was prepared by mixing 2ml of DPPH and 1ml of methanol. The absorbance was measured at 517nm in a UV-vis spectrophotometer using methanol as blank. Methanolic solution of standard ascorbic acid (0.5mg/ml) was prepared and added in range of 10-100mg/ml in test tubes containing methanol and DPPH reagent solution as a positive control.

RESULTS AND DISCUSSION

In the preliminary phytochemical analysis, it was revealed that methanolic extract was found to contain alkaloids, carbohydrates, glycosides, saponins, phenolic compounds, tannins, flavonoids, antraquinones, terpenoids, tannins, proteins and amino acids. The ethyl acetate extract of leaves were showed presence of alkaloids, carbohydrates, saponin, whereas chloroform extract tested positive for alkaloids, and carbohydrates, and the hexane extract revealed presence of carbohydrates respectively. Many plants and their extracts used against microbial infections due to the presence of secondary metabolites such as phenols (Kazmi et al., 1994). Carbohydrates content was found high (125 mg/g dw) carbohydrates (arabinose, glucose, fructose, galactose, maltose, raffinose, rhamnose, ribose, sucrose, xylulose) plays a major role in lubrication, proteins, against toxin, microbial growth stimulation (Shengjing, 2009). The chlorophyll content of *C.auriculata* is 2,86 g/mg dw. Secondary metabolite analysis is necessary for extraction, purification, separation, crystallization identification of various phyto compounds (Table-1) The methanolic extract showed higher levels of phenols (463.33 mg/g) dw than the other secondary

metabolites. The higher amount of phenol is important in regulation of plant growth, development and disease resistance. The total phenolic content of the methanolic leaf extract was 463.66±12 gallic acid equivalent phenolic compounds have redox properties, which allow them to act as antioxidant. As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as basis for rapid screening of antioxidant activity (Table-2). The in vitro antibacterial activity of methanol extract of *C. auriculata* were found to have maximum activity against all organisms. The antibacterial activity was measured by zone of inhibition.The zone of inhibition of *C. auriculata* (5mg) methanol extract was maximum against *S. aureus* (0.86± 0.2) followed by *E.coli* (0.8±0.25) and *Bacillus* (0.73±0.15), *Pseudomonas* (0.63±0.10) were observed. For the positive control, ofloxacin was, the zone ranged from 1.6± 0.2 to 2.03± 0.4 cm. The in vitro antibacterial activity of methanol extract of *C. auriculata* were found to have maximum activity against all organisms. The antibacterial activity was measured by zone of inhibition. The zone of inhibition of *C. auriculata* (5mg) methanol extract was maximum against *S. aureus* (0.86± 0.2) followed by *E.coli* (0.8±0.25) and Bacillus (0.73±0.15), pseudomonas (0.63±0.10) was observed in pseudomonas (Figure-1 and Table-3). For the positive control, ofloxacin was, the zone ranged from 1.6± 0.2 to 2.03± 0.4 cm. DPPH is one of the free radicals widely used for testing preliminary scavenging activity of the plant testing preliminary scavenging activity of the plant extract which is based on the ability of DPPH, a free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action (Raquibal Hassan et al., 2009) the DPPH assay has been largely used as a quick, reliable and reproducible parameter to search the in vitro general antioxidant activity to pure compounds as well as plant extracts (Koleva et al., 2002). The reducing capacity of compounds could serve as indicator of potential antioxidant property. In the present study, the percentage of scavenging effect on the DPPH was concomitantly increased with the increased in the concentration of both methanol leaf extract of *C. auriculata* from 10 to 100 µg/ml.

Table-1 Preliminary Phytochemical Analysis From *C.Auriculata*

Sl No	Test	Hexane	Chloroform	Ethyl Acetate	Methanol
1. Alkaloids					
a)	Mayer's Test	-	+	+	+
b)	Wagner's Test	-	+	+	+
c)	Hager's Test	-	+	+	+
2. Carbohydrates					
a)	Molisch Test	+	+	+	+
b)	Benedict's Test	+	+	+	+
3. Glycosides					
a)	Boentrager's Test	-	-	-	-
b)	Keller Killani Test	-	-	-	-
c)	Legal's Test	-	-	-	-
4.	Saponins	-	-	+	+
5. Proteins & amino acids					
a)	Millon's Test	-	-	-	+
b)	Biuret Test	-	-	-	+
c)	Ninhydrin Test	-	-	-	+
6. Phtosterol					
a)	Libermann-Bachard's Test	-	-	-	-
7. Fixed oils & fats					
a)	Spot Test	-	-	-	-
b)	Saponification Test	-	-	-	-
8. Phenolic compounds & tannins					
a)	Ferric Chloride Test	-	-	-	+
b)	Lead Acetate Test	-	-	-	+
9. Flavonoids					
a)	Alkaloid Test	-	-	-	+
10. Anthraquinone					
a)	Borntrager's Test	-	-	-	+
11. Terpenoids					
a)	Salkowski Test	-	-	-	+
12.	Tannin	-	-	-	+

Table-2 Antioxidant* activity of *Cassia auriculata

Drug	Concentration (mg/ml)	Zone of inhibition (in mm)			
		S.aureus	Pseudomonas	E.coli	Bacillus
C. auriculata	5	8.6 ± 0.2	6.3 ± 0.10	8.0 ± 0.25	7.3 ± 0.15
Ofloxacin	0.1	18.6± 0.4	20.3± 0.4	16.0 ± 0.4	18.0 ± 0.4

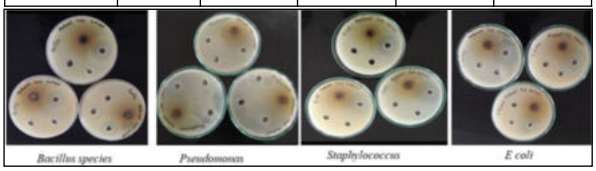

Figure-1 Anti-bacterial activity of *C.auriculata*

Table-3 Percentage Inhibition (Antibacterial Activity)

Sl No	Percentage inhibition (%)				
	10 (µg/ml)	20 (µg/ml)	60 (µg/ml)	80 (µg/ml)	100 (µg/ml)
1.Methanolic extract	4.9±2.41	21.31±1.23	44.26±0.01	63.93±2.32	78.68±5.60
2.Ascorbic acid	37.70±0.1	85.24±3.40	98.36±2.10	98.36±0.13	98.36±0.00

CONCLUSION

In the phytochemical screening of the leaf extract, we found maximum recovery of phytochemicals in methanolic extract compared to the other solvents used. In the preliminary analysis we found it to be positive for most of the phytochemicals in methanolic extract and showed absence of fat, oils and glycosides. Quantitative analysis of primary metabolites in leaves are being reported for the first time as earlier studies were done in flowers, root and bark. Antibacterial and antioxidant activity of *C. auriculata* can be further studied in the treatment of cancer and infectious diseases. There is a scope for further research in this area to determine the phytochemicals responsible for pharmacological activities.

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