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Research Article

PHYTOCHEMICAL ANALYSIS, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *CASSIA AURICULATA*

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Abstract: Most of the fast research indicated that plants are a good source in treating many of the diseases affecting mankind. In this notice we successfully screened out *Cassia auriculata* for the phytochemical analysis, antimicrobial activity and antioxidant activity. Aqueous extract of this plant were discovered in the phytochemical analysis and results indicated that extract have high amount of steroids, tri terpenoids, tannins, flavonoids, alkaloids, saponins, glycosides, gums and mucilage and phenolic compounds whereas absence of carbohydrates. Antimicrobial screening for the four bacterial strains like *Pseudomonas aeruginosa*, *E. coli*, *Staphylococcus aureus* and *Bacillus subtilis* were performed in which good antimicrobial activity was associated with the *Bacillus subtilis* followed by *Staphylococcus aureus*. Antioxidant activity was performed in terms of inhibition of free radicals by DPPH. The percentage of this activity was in the range of 70-90% by comparing the standard ascorbic acid. Based on these properties, it was considered as good medicinal plant to treat the all diseases of humans.

Keywords: *Cassia auriculata*, phytochemical analysis, antimicrobial activity, DPPH, antioxidant activity

INTRODUCTION

Since millions of years nature has been a good source to prevent several diseases. Use of herbs is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals. The use of plants as medicines predates written in human history. According to the WHO, 80% of the populations were mainly dependent on traditional medicine for their primary health care. Now it's the time to screen for the medicinally important plant for their pharmaceutical use (Benignant *et al.*, 1996[1] Multi Drug resistance in recent years is due to indiscriminate use of synthetic antimicrobial agents for the treatment of diseases gave an idea to researchers to trace out plant compound to treat diseases (Charaka and Sofovora Basu, 2000) The use of, and search for, drugs and dietary supplements derived from plants have accelerated in recent years. The present literature and ethno botanical information revealed that plants were sleeping giants of pharmaceutical industry and consumption of nontoxic, easily affordable plant to human kind is very essential (Jayashree and Maneemegalai, 2008). Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities (Meretrk *et al.*, 2006). Diseases like malaria, epilepsy, infantile convulsion, and diarrhea, dysentery, fungal and bacterial diseases were traditionally managed by medicinal plants. The bio active compounds that are present in the herbal plants are mainly involved in the blocking of the oxidative stress, thereby increasing the age of the human beings.

Cassia auriculata is a natural herb belongs to family Fabaceae and locally called as 'avaram'. It is shrub, having smooth bark and mostly seen in the India, Sri Lanka and other Asian countries. These leaves are mainly used to treat anthelmentic infections, ulcer, leprosy and other skin diseases. Urinary discharge, diabetes and throat infections were treated by flowers of this plant. The seed mainly eliminates the diseases like dysentery and conjunctivitis. The bark also used to reduce the astringent.

In the present investigation we have reported the isolation of bioactive compounds from aqueous extract and we have also seen their phytochemical analysis. From those isolated compound antimicrobial and antioxidant analysis were carried out.

MATERIALS AND METHODS

Collection and extraction of plant materials:

The flowers of *Cassia auriculata* are used in this study were collected from Seshachalam forests of Chittoor district, Andhra Pradesh, India. These flowers are dried in shade condition and made it into powder, and then this extracted dark colored powder is mixed with water to molten mass using rotary vacuum evaporator.

Phytochemical analysis

Phytochemical analysis were carried out in quantitatively to see the presence of active ingredients like steroids, triterpenes, tannins, flavonoids, saponins, alkaloids, carbohydrates, gums, Mucilage, Phenolic

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compounds and glycosides (Harborne,1998) .

Antimicrobial activity

Tested microorganisms: *Pseudomonas aeruginosa*, *E. coli*, *Staphylococcus aureus* and *Bacillus subtilis* (obtained from IMTECH, Chandigarh India) are used for antimicrobial activity, Prior to that antimicrobial testing, organisms were sub cultured at 24 hours.

Antimicrobial activity: Active sub cultured organisms were transferred into an agar plate containing 20 ml of nutrient agar media. Disc diffusion method (Bauer *et al.*, 1966) was performed normally in which the discs were made into 6mm diameter steeped with known concentration of the standard drugs and extract was placed on the surface of the agar in Petri plate for bacterial strains and were scattered with 100µl of microbial culture (5x105 CFU/ml). The plates with standard drugs and extract were incubated for 24 hours at 35±2.

Minimum inhibitory concentration (MIC): Micro broth dilution method is the common method used in minimum inhibitory concentration in which 96 well micro titer plates was used (Cruickshank, 1968 and NCCLS, 1999). The different concentrations of aqueous extract ranging from 0.1 to 2mg/ml were evaluated for their MIC value and 0.1 ml of standard inoculums (105 CFU/ml) were added to each well. The incubated plates were examined with ELISA reader (TECAN, Sunrise, and China) at 620nm. The study performed in triplicate and mean values was noted.

Antioxidant activity:

DPPH radical scavenger activity: By using 2, 2-diphenly-1-picrylhydrazyl, the antioxidant activity was determined to see the antioxidant substances in the aqueous extract of plant (Burits and Bucar, 1999). The 1 ml of different concentrations of the plant extract were added to 4ml of 0.004% methanol solution of DPPH, after proper incubation at the room temperature the absorbance was read at 517nm against blank. The antioxidant activity in percentage (I %) were calculated by using the below formula,

$$I\% = [(A \text{ control} - A \text{ sample}/A \text{ control})] \times 100$$

Where, a control is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound.

Reducing power assay: The reducing power of aqueous extract was determined by the procedure of Oyaizu *et al.*, (2003)[7]. The methanolic solution of extract was mixed with 0.2M phosphate buffer and 1% potassium ferricyanide in 2.5 ml and 2.5 ml respectively. The mixture was incubated then 2.5ml (10%) trichloroacetic acid was added which was then centrifuged at 3000rpm for 10 min. The supernatant was mixed with distilled water (2.5ml) and 0.1% of 0.5 ml Fecl3. The absorbance was measured at 700nm against blank and values were measured by comparing the standard Ascorbic acid.

RESULTS

Phytochemical analysis:

In table 1 it table describes secondary metabolites and other phytochemicals in the aqueous extract. + indicates presence of active compounds and – describes absence of active compounds.

S.No	Phytochemicals	Aqueous Extract
1	Steroids	+
2	Tri terpenoids	+
3	Tannins	+
4	Flavonoids	+
5	Alkaloids	+
6	Saponins	+
7	Glycosides	+
8	Gums and Mucilage	+
9	Carbohydrates	--
10	Phenolic compounds	+

Phytochemical analysis of the aqueous extract revealed that presence of bioactive compounds and the results summarized in the table 1. The present study plant contains high amount of steroids, tri terpenoids, tannins, flavonoids, alkaloids, saponins, glycosides, gums and mucilage and phenolic compounds whereas absence of carbohydrates. This presence or absence of the bioactive compounds decides the medical property of the plant to use for health.

Antimicrobial activity:

The present table 2 indicates the antimicrobial activity in which zone of inhibition in mm and MIC in mgs

Organism Name	Inhibition Zone at 20µg/ml	Inhibition Zone at 40µg/ml	Inhibition Zone at 60µg/ml	Inhibition Zone at 80µg/ml	MIC
Pseudomonas aeruginosa	06.43±0.2	07.67±0.2 8	09.49±0.2	11.22±0.8	15.57±0.7
E. coli	06.13±0.4	08.17±0.2	10.19±0.8	12.12±0.1	12.34±0.8
Staphylococcus aureus	9.56±0.5	11.56±0.2	13.46±0.9	14.94±0.7	16.14± 0.4
Bacillus subtilis	11.62 ±1.5	13.55±1.0	15.62±1.2	16.33±0.8	11.23±1.6

The organisms used in this present study to see the antimicrobial activity were including *Pseudomonas aeruginosa*, *E. coli*, *Staphylococcus aureus*, *Bacillus subtilis*. Zone of inhibition and minimum inhibition concentration of different organisms were summarized in the table 2. The aqueous extracts of plant ranging from 20-80 µg/ml were used to detect the antibacterial activity by comparing the standard drugs like ampicillin (20µg/ml), tetracycline (20µg/ml) and ketonazole (20µg/ml). The present results clearly revealed that the plant extract showed good antimicrobial activity

against *Bacillus subtilis* (The values at different concentrations were 11.62±1.5, 13.55±1.0, 15.62±1.2 and 16.33±0.8) followed by *Staphylococcus aureus*. The moderate antimicrobial activity was observed against *E. coli*. *Pseudomonas aeruginosa* was associated with lower antimicrobial activity (The values at different concentrations were 06.43±0.2, 07.67±0.2 8, 09.49±0.2 and 11.22±0.8). The MIC values of different organisms varied differently.

Antioxidant assay:

Antioxidant activity of the plant extract showed good results by increasing the concentration of the compounds (100, 200, 300, 400, and 500µg/ml).The average percentage of this activity within 70-90% by comparing the standard drug Ascorbic acid. Normally ascorbic acid showed highest antioxidant activity at all concentrations (Fig.1). Secondary metabolites such as polyphenols and flavonoids mainly associated with this antioxidant property of the compound.

Table.3: Results of antioxidant activity of *Cassia auriculata* aqueous extract.

	% Anti-oxidant activity	
Concentration (µg/ml)	Aqueous extract	Ascorbic acid
100	65.12± 0.004	78.55 ± 0.003
200	67.67 ± 0.050	84.32 ± 0.001
300	77.56 ± 0.003*	88.03 ± 0.002
400	85.43 ± 0.002*	94.55 ± 0.003
500	87.60 ± 0.001*	95.87± 0.002

*P<0.05 no significant difference

DISCUSSION

Plants have so many biologically active material which were still to be discovered and analyzed (Gislene *et al.*, 2000). By this aspect *Cassia auriculata* was screened for the presence of steroids, tri terpenoids, tannins, flavonoids, alkaloids, saponins, glycosides, gums and mucilage, phenolic compounds and carbohydrates in which carbohydrates where not at all present in the plant. Fast research studies indicated that flavonoids mainly associated with the several health benefits like antimicrobial activity (Narayana *et al.*, 2001), anti-inflammatory and anti-tumor (Castillo *et al.*, 1989). Glycosides are very much known to have ability to reduce the antiseptic properties (Robbinson, 1967). Membranes are mainly disintegrated with saponins as having activity of reducing the lower surface tension and emulsifying activity. These proved to be associated with the antimicrobial activity. Inactivation of microbial adhesion enzymes, cell envelop transport proteins and also involved in wound healing activity (Natarajan *et al.*, 2005).

The antimicrobial activity were carried out by minimum inhibitory concentration (MIC) and disc diffusion method in which plant extract showed good antimicrobial activity. The aqueous extract of plant has ability to kill both gram positive and gram negative bacteria, there by confirmed it as a good antibacterial agent. Bio active compounds having good antimicrobial properties where isolated from plants but most of the compounds chemical structures were not clearly analyzed as antibiotics (Gibbons, 2004).

Antioxidants that block the reactive oxygen species may be involved in preventing oxidative diseases like cardiovascular diseases, neurovascular diseases and autoimmune diseases. The present plant extract was also great value to prevent these diseases as it has good antioxidant property.

CONCLUSION

This present study evaluated the presence of various biologically active metabolites in the aqueous extract of *Cassia auriculata* and these metabolites were associated with antimicrobial and antioxidant activity.

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