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Bactericidal Activity of Extracts of Different Flowering Stages of Cassia Auriculata and Screening of its Amino Acids

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Abstract: *Cassia auriculata*is an Indian medicinal plant which is known for its various therapeutic properties. In this study, the bactericidal activity of the different flowering stages of the *Cassia auriculata*bud, before seedling and dried stages were analysed after extraction with different solvents like DMSO, methanol and water. Different concentrations of potential methanol extracts of the three flowering stages were found to be active against ten different human pathogens. The highest activity was observed by methanol extract of the fresh flowers with 19 and 18mm of inhibition zone against *Proteus mirabilis* and *Staphylococcus aureus* respectively. Antioxidant property of the methanol extract was checked and was found to be moderately effective. Presence of some conditionally essential amino acids like Arginine, cysteine, glutamine, glycine, proline and tyrosine and essential amino acids like histidine, lysine, threonine, methionine, isoleucine and tryptophane were screened for its presence in the methanol extracts of all three stages of the flowers and 4 conditionally essential amino acids and 3 essential amino acids were detected using paper chromatography. Hence it can be concluded that the fresh flowers of the *Cassia auriculata* can serve as a potential source for drug development.

Key words: *Cassia auriculata* · Bactericidal Activity · Paper Chromatography · Antioxidant Activity · Amino Acids

INTRODUCTION

Infectious diseases are the leading cause of death throughout the world. Antibiotic resistance has become a global concern. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens [1]. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases.

Cassia auriculata commonly known as tanners cassia, also known as “avaram” in Tamil language is a shrub belonging to the Caesalpiniaceae family. The shrub is especially famous for its attractive yellow flowers which are used in the treatment of skin disorders and body odour. It is widely used in traditional medicine for rheumatism, conjunctivitis and diabetes. It has many medicinal properties. Its bark is used as an astringent, leaves and fruits anthelminthic, seeds are used to treat eye troubles and root employed in skin diseases [2]. It is also used for the treatment of ulcers, leprosy and liver disease [3]. The antidiabetic, hypolipidemic [4] and antioxidant [5] and hepatoprotective [6] effects of *Cassia auriculata*have been reported. It was also observed that flower and leaf extract of *Cassia auriculata*showed antipyretic activity [7].

In recent years, secondary plant metabolites (phytochemicals) previously with unknown pharmacological activities have been extensively investigated as a source of medicinal agents. Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes [8].

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In the present study, the phytochemical nature of *Cassia auriculata* was experimented by antibacterial assay of the various solvent extracts at different flowering stages, presence of important amino acids were screened by paper chromatography, antioxidant property of the methanol extract was analysed and the phenolic compounds and flavonoids were estimated.

MATERIALS AND METHODS

Collection of Samples: *Cassia auriculata* plants were collected in and around Chidambaram having buds, fresh and dried flowers to extract the bioactive compounds. The samples were washed with distilled water to clean the adhering dust particles.

Preparation of Solvent Extracts: Sufficient and equal grams of samples were cut into small pieces and placed in three 250 ml conical flasks containing methanol, DMSO and water for solvent extraction of the bioactive compounds.

Bacterial Strains: Bacterial strains employed for the test which included were *Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumonia, Enterococcus fecalis, Proteus vulgaris, Vibrio cholera* and *Aeromonashydrophila*obtained from Department of Microbiology, Annamalai University.

Soxhlet Extraction: 20 to 30 grams of each medicinal plant samples were cut into small pieces and further grinded, placed in the soxhlet extractor for the extraction of bioactive compounds [9]. Methanol was used to extract the bioactive components of the medicinal plant samples. Before extraction of the bioactive compounds, the samples were flushed with organic solvents such as DMSO and Methanol for two times. Thus collected extracts were concentrated by exposing them in a laminar air flow and stored at 4°C until further use.

Preparation of Sterile Antibiotic Disc: Sterile antibiotic disc were obtained by using Whatmann no.1 filter paper. Samples were incorporated into sterile disc. Each sterile disc was incorporated individually with 200 to 400 µl of the extract using micropipette. This was achieved by adding small quantities of extract and the discs were allowed to dry in laminar air flow.

Preparation of Cultures for the Assay: 10 ml of nutrient broth were prepared in the test tubes. The nutrient broth were inoculated with the given bacterial strains under aseptic conditions and incubated at 37°C for 18-24 hours.

Assay of Antibacterial Activity: Assay of the antibacterial activity of the medicinal plant extracts was done by disc diffusion technique [10]. The nutrient agar plates were prepared and the test bacterial strain was smeared on the nutrient agar surface using sterile cotton swab. The antibiotic discs loaded with plant extracts were placed on the surface of the nutrient agar plates. Controls were maintained by loading Dimethyl sulfoxide, methanol and distilled water on discs. Then the plates were incubated at 37°C for 18 to 24 hours. The inhibition zone formation was observed and recorded.

Antioxidant Activity: Total antioxidant activity of the methanol extract of fresh flower was measured [11]. 7.45ml sulphuric acid (0.6M solution), 0.9942g sodium sulphate (28mM solution) and 1.2359g ammonium molybdate (4mM) were mixed together in 250ml distilled water and labelled as Total Antioxidant Capacity (TAC) reagent. 100µl of the extract was dissolved in 1ml of TAC reagent. Distilled water was used as blank. The tubes were capped and incubated in a boiling water bath at 95°C for 90 min. Absorbance was measured at 695nm in a spectrophotometer and plotted in a graph.

Detection of Amino Acids: Whatmann No. 1 filter paper was used for paper chromatography. Amino acids kit (CHH laboratory reagent, Cabell Huntington hospital, Huntington) was used for standard amino acids. Three extracts were prepared by using pre-weighed quantity of powdered material in a known volume of water, 9% (w/v) aqueous sodium chloride solution and ethanol. Conditionally essential amino acids like arginine, cysteine, glutamine, glycine, praline and tyrosine and essential amino acid like histidine, lysine, threonine, methionine, isoleucine and tryptophane were used as standards for detecting its presence in the different methanolic extracts. The experimental extracts were spotted on the chromatographic paper along with standards samples. The mobile phase was allowed to run to a certain height and the chromatogram was dried at room temperature. Amino acids were detected by using ninhydrin spray on the spotted Whatmann filter paper. The Rf values of the amino acids of the experimental samples were determined and compared with the standards.

RESULTS AND DISCUSSION

Plants are an important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the in vitro antimicrobial activity assay and in recent years several reports are available on antimicrobial activity of plant extracts on human pathogenic microorganisms [12].

Anushia*et al.* [13] checked the antibacterial and antioxidant activities in *Cassia auriculata* and found that the methanol extract is effective against two of the tested organisms i.e., *S. aureus*and *E. coli* both at the concentration of 64mg/ml. In the present study, methanol extracts *Cassia auriculata*budshowed significant zone of inhibition against *Escherichia coli*, *Enterococcus fecalis*and *Staphylococcus aureus* at 100 ppm concentration (Table 1).

Among these methanolic extracts the maximum inhibitory activity was obtained in fresh flowers of *Cassia auriculata* against the pathogens like *Proteus mirabilis* and *Staphylococcus aureus* with maximum zones of inhibition of 19 and 18mm respectively (Table 2).

Studies on the antibacterial activity of ethanol, methanol and aqueous extracts of dry flower and ethanol, methanol and acetone extracts of fresh flower of *Cassia auriculata*was conducted using agar disc diffusion method. Maneemegalai and Naveen [14] studied the antibacterial activity of the microorganisms; *Staphylococcus aureus, Enterococcus fecalis, Bacillus subtilis, Salmonella typhi, Salmonella paratyphi A, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiellapneumoniae, Vibrio cholerae*and *Shigelladysenteriae.* The maximum activity was observed against all organisms except *Pseudomonas aeruginosa*and *Klebsiellapneumoniae*. Presence of phytochemicals such as terpenoids, tannins, flavonoids, saponin, cardiac glycosides and steroids were observed and they declared that *Cassia auriculata*is observed to have antibacterial activity and can be used for medicinal purposes.

Perumalsamy and Ignacimuthu [15] reported that the leaf extracts of *Cassia auriculata*exhibited significant broad spectrum activity against *Bacillus subtilis*and *Staphylococcus aureus*. Antimicrobial activity of *Cassia auriculata*flower extract has been observed by Narayanan *et al.* [16]. The extract of *Cassia auriculata*was found to have potent microbicidal activity against the *E. coli* in poultry [17]. In this study ethanol and methanol extracts of dry and fresh flowers, aqueous

Table 1: Antibacterial activity of methanolic extracts of *Cassia auriculata*bud

		Zone of inhibition (mm)		
		Concentration in ppm		
S. No.	Organism	50	100	200
1.	*Pseudomonas aeruginosa*	-	10	12
2.	*Proteus mirabilis*	11	12	12
3.	*Escherichia coli*	12	13	15
4.	*Staphylococcus aureus*	13	15	14
5.	*Salmonella typhi*	10	11	12
6.	*Klebsiella pneumonia*	11	11	13
7.	*Enterococcus fecalis*	12	14	14
8.	*Proteus vulgaris*	10	12	12
9.	*Vibrio cholera*	8	9	10
10.	*Aeromonashydrophila*	11	12	13

Table 2: Antibacterial activity of methanolic extracts of *Cassia auriculata*fresh flowers

		Zone of inhibition (mm)		
		Concentration in ppm		
S. No.	Organism	50	100	200
1.	*Pseudomonas aeruginosa*	12	12	13
2.	*Proteus mirabilis*	16	17	19
3.	*Escherichia coli*	15	16	16
4.	*Staphylococcus aureus*	16	17	18
5.	*Salmonella typhi*	14	14	15
6.	*Klebsiella pneumonia*	11	11	13
7.	*Enterococcus fecalis*	12	14	15
8.	*Proteus vulgaris*	10	12	15
9.	*Vibrio cholera*	12	12	13
10.	*Aeromonashydrophila*	13	13	16

Table 3: Antibacterial activity of methanolic extracts of *Cassia auriculata*before seedling stage

		Zone of inhibition (mm)		
		Concentration in ppm		
S. No.	Organism	50	100	200
1.	*Pseudomonas aeruginosa*	7	8	8
2.	*Proteus mirabilis*	6	7	9
3.	*Escherichia coli*	11	13	15
4.	*Staphylococcus aureus*	8	10	12
5.	*Salmonella typhi*	7	11	13
6.	*Klebsiella pneumonia*	11	11	13
7.	*Enterococcus fecalis*	12	14	15
8.	*Proteus vulgaris*	10	12	12
9.	*Vibrio cholera*	12	12	14
10.	*Aeromonashydrophila*	7	10	11

Table 4: Antibacterial activity of methanolic extracts of *Cassia auriculata*dried flowers

S. No.	Organism	Zone of inhibition (mm) Concentration in ppm 50	100	200
1.	*Pseudomonas aeruginosa*	8	9	10
2.	*Proteus mirabilis*	10	8	11
3.	*Escherichia coli*	10	13	15
4.	*Staphylococcus aureus*	8	10	12
5.	*Salmonella typhi*	7	10	13
6.	*Klebsiella pneumonia*	11	11	12
7.	*Enterococcus fecalis*	12	13	14
8.	*Proteus vulgaris*	10	10	12
9.	*Vibrio cholera*	12	12	13
10.	*Aeromonashydrophila*	7	10	10

Table 5: Amino acids detected in fresh flowers of *Cassia auriculata*

S. No.	Name of the amino acids	Rf values Std. Amino acids	Amino acids in flowers
1.	Cysteine	0.40	0.30
2.	Proline	0.43	0.40
3.	Arginine	0.20	0.20
4.	Glutamine	0.13	0.14
5.	Histidine	0.11	0.10
6.	Lysine	0.14	0.13
7.	Isoleucine	0.72	0.72

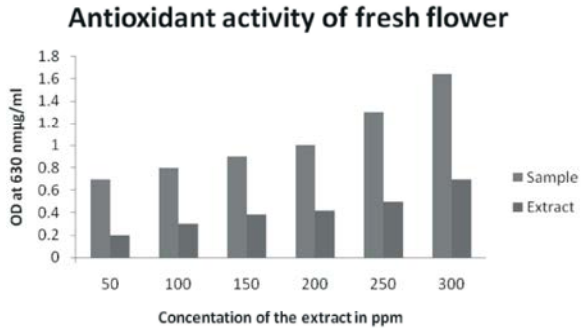

Fig. 1: Antioxidant activity of methanolic extract of fresh flowers

extract of dry flower and acetone extract of fresh flower was found to have higher inhibitory activities against *Enterococcus fecalis, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Proteus mirabilis, Salmonella typhi, Salmonella paratyphi A, Vibrio cholerae*and *Shigelladysenteriae*when compared to *Pseudomonas aeruginosa*and *Klebsiellapneumoniae*.

Antioxidant Activity: Free radical scavenging properties of the methanol extract was assessed using total antioxidant method by using ascorbic acid as standard. The activity increased with increase in concentration of the sample (Fig. 2). When compared with standard ascorbic acid (1.64µg/ml) the extract showed lesser activity (0.70µg/ml).

Paper Chromatography for Amino Acids Detection: Qualitative determination of amino acids from chromatogram specified glutamine, cysteine, proline, arginine, leucine, isoleucine and histidine were observed minor quantities using mobile phase butanol: acetic acid: water (6:4:2) and n-butanol: ethanol-water-pyridine-ammonia (4:2:1:2:1). Totally seven amino acids were detected in chromatogram and others were present only in negligible amount.

Gaikwad *et al.* [18], studied the amino acids present in the *Cassia auriculata* using Qualitative determination of amino acids from chromatogram and observed L-Cystine, DL-Alanine, L-Proline, L-Leucine, DL-Isoleucine methionine as major constituents and D-Threonine, L-Ornithrine hydrochloride, Aspartic acid, Hydroxyproline and Glycine as minor constituents using mobile phase butanol: acetic acid: water (6:4:2) and n-butanol ethanol-water-pyridine-ammonia (4:2:1:2:1). The remaining amino acids were in negligible amount. Totally ten amino acids were present. Among these amino acids L-Leucine, D-Threonine, DL-Isoleucine methionine are essential amino acids whereas L-Cystine, DL-Alanine, Lproline, Glycine, Hydroxyproline, Aspartic acid, L-Ornithine hydrochloride are non-essential amino acids [19].

On the basis of the results obtained in this present investigation it can be concluded that the methanol extract of *Cassia auriculata* bud, fresh flowers and dry flowers have significant in vitro antimicrobial activity. The present observation suggests that the methanolic extract of *Cassia auriculata*flowers at fresh stage seems suitable to verify the amino acids presence and antioxidant properties.

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