

EVALUATION OF ANTIBACTERIAL ACTIVITY OF CASSIA AUSTRALIS LINN

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Cassia australis Linn is commonly called as seemia, which belongs to Leguminosae family. It is an ancient medicinal shrub with an extensive ethnobotanical history in Asia (Singh and Pandey, 1998). It has been used for medicinal purposes for centuries in India and other countries. The leaves being an excellent laxative and febrifuge have been used in the treatment of anemia, typhoid, cholera, tumors and probably leprosy, which are commonly used by the aboriginal people (Kirtikar and Basu, 1975 and Wealth of India). In spite of its reported use in infectious diseases, no systematic studies of its antimicrobial activity have been reported. Hence an effort has been made to establish the antibacterial activity of various extracts of experimental plant leaves against Gram positive and Gram negative bacterial strains. Additionally in present work, we report the preliminary phytochemical and physicochemical investigations of various extracts.

Material and methods-The plant was collected during the month of November 2006 from Satpuda Mountain of Nandurbar district of Maharashtra. The specimen has been deposited in the department of Botany, R. C. Patel A. S. C. College, Shirpur, Dist. Dhule (M.S.). The leaves of *Cassia australis* were used for present study.

Microbial Culture-The microbial culture like *Escherichia coli* (NCIM 2065), *Staphylococcus aureus* (NCIM 5021), *Pseudomonas aeruginosa* (NCIM 2200), *Proteus vulgaris* (NCIM 2027) and *Bacillus subtilis* (NCIM 2063), were procured from National Collection of Industrial Microorganisms, NCL, Pune. The medium Nutrient agar and reference standard antibiotic (ampicillin) were obtained from Hi-media laboratories Ltd. Mumbai.

Preparation of Plant Extract-Freshly collected leaves were dried in shade and coarsely powdered. A 250 gm of powder was extracted in an aspirated bottle with various solvents like aqueous, hydroalcohol (1:1), methanol, petroleum ether and chloroform by cold

maceration for 48 hrs. These extracts after maceration were filtered through Whatmann filter paper No.1, evaporated on water bath and finally dried in vacuum on rotary evaporator (Bhavani et al., 2006). The residue was diluted with Dimethyl sulfoxide (DMSO) to get final concentrations of 1000, 2000 and 3000 µg/ml used for evaluation of antibacterial properties.

Preliminary Phytochemical study-The individual extracts were analyzed for their phytochemical composition by qualitative method. Dragendorff's reagent and Mayer's reagent were used to know the presence of alkaloids and cyanogenic glycosides using cold concentrated sulphuric acid test. Extracts were tested for phenolics using Folin Ciocalteu reagent. Flavonoids were detected by appearance of effluences with pink color by dissolving plant extract in 10 % HCL and Zinc powder. Extracts were also tested for terpenoids and saponins. Tannins were detected using gelatin salt block test (Harborne, 1984 and Wagner, 1984).

Physicochemical study-Dry powdered form of leaves of *C. australis* was subjected for the determination of physicochemical studies of ash value as per African Pharmacopoeias. The objective of ashing is to remove all traces of organic materials interfering in analysis of inorganic elements. The ash was subjected to qualitative estimation of various inorganic elements like iron, sulphate, chloride, zinc, sodium, calcium, magnesium, copper (Kokate, 1994) etc.

Antibacterial activity-The antibacterial activity of all above mentioned extracts was evaluated by employing 24 hrs old microbial cultures using agar well diffusion method. DMSO was used as a solvent for the dissolution of the different extracts. The medium was sterilized by autoclaving. About 25 ml of molten nutrient agar medium was transferred aseptically into each sterilized plate (10 cm diameter). The sterile Petri dishes containing solidified nutrient agar medium were aseptically inoculated with respective microbial strains by spreading to get a bacterial lawn. In each plate 2

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wells of 8 mm diameter were made using a sterile cork borer. Accurately 100 μ l test samples of various extracts of plant at different concentration and standard antibiotic solution (ampicillin) were transferred to cups aseptically. The plates were then maintained in refrigerator for about 20 minutes to allow the diffusion of plant extracts and standard solution into medium. Plates were incubated at 37°C for 24 hrs (Pelczar, 1974 and Shastry, 2005). Diameter of zone of inhibition was measured and compared with that of standard.

Result and Discussion—The preliminary phytochemical analysis of the leaf extracts revealed the presence of alkaloids, flavonoids, steroids, tannins, phenolics, glycosides and terpenoids as presented in Table 1. The physical constants such as total ash value (07.50%), acid insoluble ash (0.53%), water soluble ash (0.93%), moisture content (13.34%) and extractive values are specific identification (Table 2A). The soluble extractive values with different solvents such as aqueous, hydroalcoholic, methanol, petroleum ether and chloroform were found to be 31.8%, 21.5%, 11.7%, 4.6% and 2.9% respectively, which indicates the nature of constituents present in it (Table 2B). In physicochemical screening iron, sulphate chloride, Zinc and sodium ions were detected (Table 3). The powdered leaves of plant on various chemical reagents

showed different characteristics as shown in Table 4.

The results obtained from the disc diffusion assay (Table 5) showed that there has been an increasing effect on bacterial growth inhibition with increasing concentration of the extract and the extract showed good inhibitory activity on almost all the bacteria tested. Aqueous and petroleum ether extract showed strong antibacterial activity. The result concluded that, the *Cassia australis* has exhibited potent antibacterial action against *Bacillus subtilis*, *Staphylococcus aureus* and *Proteus vulgaris*. The rest of microbial strains showed moderate susceptibility to the extracts. The antibacterial potential of these extracts needs to be further investigation, to find out active principle of phytoconstituent in given plant, which is responsible for antibacterial activity. For confirmation of the proposed hypothesis, systematic scientific study should be carried out in future.

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Table 1 Phytochemical investigations of *C. australis* leave

Phytoconstituent	Plant Extract				
	Aqueous	Hydroalcoholic	Methanol	Petroleum ether	Chloroform
Alkaloids	-	-	-	-	+
Flavonoids	+	+	-	+	-
Steroids	+	+	+	+	+
Tannins	+	+	-	-	+
Phenolics	+	+	-	-	-
Glycosides	+	+	-	-	-
Terpenoids	+	-	+	-	-
Saponins	-	-	-	-	-

+ = Present, - = Absent

Table 2(A) Ash values of *C. australis* leave

Type of ash value	Percentage (w/w)
Total ash	7.5
Water soluble ash	0.93
Acid insoluble ash	0.53
Moisture content	13.34

Table 2(B) Extractive values of *C. australis* leave

Type of Extracts	Percentage (w/w)
Aqueous	31.8
Hydroalcoholic	21.5
Methanol	11.7
Petroleum ether	4.6
Chloroform	2.9

Table 3 Details of qualitative Physicochemical Test

Inorganic Elements	Inference
Calcium	-
Magnesium	-
Iron	+
Sulphate	+
Chloride	+
Zinc	+
Copper	-
Sodium	+

+ = Present, - = Absent

Table 4 Behavior of powder with different chemical reagents

Reagent	Behavior
Conc. HNO ₃	Light yellow
Conc. H ₂ SO ₄	Red brown
Conc. HCL	Bottle green
Acetic acid	Yellowish green
NaCl	Green
NH ₄ OH	Green
NaOH	Yellow
KOH	Yellow

Table 5 Antibacterial activity of various extracts of Cassia australis L.

Plant Extract	Concentration (µg/ml)	Zone of Inhibition in mm				
		<i>P. vulgaris</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>P. auriginosa</i>
Aqueous Extract	1000	—	07.50±0.65	—	07.22±0.44	06.04±0.49
	2000	08.85±0.56	10.68±0.55	07.00±0.35	08.68±0.77	09.76±0.26
	3000	11.89±0.74	12.06±0.90	09.66±1.08	11.82±0.66	11.22±0.36
Hydroalcoholic Extract	1000	—	—	—	08.77±0.55	08.65±0.60
	2000	—	09.86±0.88	—	10.68±0.55	10.72±0.46
	3000	10.72±0.46	14.33±0.65	10.08±0.84	12.06±0.90	13.06±0.38
Methanol Extract	1000	07.50±0.65	—	—	08.85±0.56	—
	2000	08.77±0.55	—	09.86±0.88	09.86±0.88	—
	3000	12.10±0.25	09.30±0.33	11.24±0.87	12.59±0.50	09.76±0.26
Petroleumether Extract	1000	—	09.82±0.05	—	—	09.62±0.55
	2000	06.89±0.22	10.55±0.94	08.59±0.61	09.86±0.88	11.89±0.74
	3000	09.76±0.26	12.59±0.50	10.34±0.50	12.94±0.80	13.33±0.66
Chloroform Extract	1000	—	—	—	—	06.90±0.43
	2000	05.53±0.65	08.53±0.74	—	—	08.68±0.77
	3000	08.68±0.77	11.32±0.06	08.90±0.65	10.55±0.94	13.26±0.24
Ampicillin	100	19.22±0.37	23.20±0.50	16.70±0.60	19.50±0.25	18.90±0.47

Values are expressed as mean ± S.D., n=6 determinations.

Solvent (DMSO) did not show any zone of inhibition; - Zone was not develop

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