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PHARMACOGNOSTIC EVALUATION OF *CHONEMORPHA GRANDIFLORA*, AN ENDANGERED MEDICINAL PLANT

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ABSTRACT

Chonemorpha grandiflora, (Roth) M.R. and S.M. Almeida syn *C. fragrans* (Moon), Alston (Apocynaceae) has been included in the list of an endangered medicinal plants. It is used in medicinal preparations like kumaryasavam and sudarsanasavum used as tonic[3]. Entire plant, roots and root bark are used for fever and stomach disorders. The plant is useful in treatment of skin diseases and inflammations. Phytochemical investigations were carried out using *in vivo* and *in vitro* plant material of *C. grandiflora* collected from Dandeli, Uttara Kannada district, Karnataka state. Percentage of total alkaloids was found to be more in stem with bark as compared to the leaves whereas percentage of total alkaloids in callus was found to be more as compared to *in vitro* plantlets. TLC studies revealed different alkaloid profile in callus as compared to *in vitro* plantlets. HPLC analysis revealed the presence of camptothecin, an important anticancer compound in the ethanolic extracts of stem with bark of *Chonemorpha grandiflora* from Thrissur district, Kerala state (Kulkarni *et al,* 2010). The presence of camptothecin in the ethanolic extracts of stem with bark of *Chonemorpha grandiflora* from Dandeli, Karnataka state has been confirmed during the present studies. The amount of camptothecin calculated for stem with bark was 0.012 mg/g and 0.007 mg/g for the leaves. Ethanolic extracts of stem with bark and callus were tested for their antimicrobial activity. Callus extracts exhibited good antimicrobial activity against gram positive organism comparable with that of cephotaxime, the standard antibiotic.

INTRODUCTION: *Chonemorpha. grandiflora* (Roth) M.R. and S. M. Almeida syn. *C. fragrans* syn. *C. macrophylla* is a medicinal plant [1, 2] used in Indian medicinal systems. Entire plant, roots and the root bark are used for fever and stomach disorders. The trade is mainly confined to Kerala state under the name Perumkurumba and the dried roots are sold commercially [3]. In Mizoram, the plant is used for treatment of gynaecological disorders [4] . *C. grandiflora,* has been included in the list of threatened medicinal plants [5]. It is known to possess muscle relaxant and antiparasitic properties [6, 7]. Phytochemical analysis of *C. grandiflora* has revealed the presence of steroidal alkaloids, like chonemorphine and funtumafrine [8, 9]. Camptothecin, a well known anticancer compound has been reported by us from the ethanolic extracts of the stem bark of *C. grandiflora* collected from Kerala state and also in the callus cultures of *C. grandiflora* [10]. So far, there are no reports on antimicrobial activity and there are scanty reports on secondary metabolite

production *in vivo* and *in vitro* for this plant. Therefore, *C. grandiflora* was selected for the present investigations.

MATERIAL AND METHODS

Collection of Plant Material: The plant material of *Chonemorpha grandiflora* was procured from Dandeli, Uttar Kannada district, Karnataka state, India. The plants were identified and deposited at Botanical Survey of India, Western circle, Pune, India. The stem with stem bark and leaves were separated and shade dried at room temperature to constant moisture content. The dried plant material was powdered and stored in plastic bottles till further use.

***In vitro* Studies:** Nodal sectors of *C. grandiflora* were grown on Murashige and Skoog's medium(MS medium) [11] supplemented with 8.8 µM BAP to obtain *in vitro* shoots. Leaf explants were grown on MS medium supplemented with 4.52 µM 2, 4- D to establish callus. *In vitro* plantlets and leaf callus were dried, powdered and used for preparation of extracts for phytochemical analysis and studies on antimicrobial activity.

Preparation of Extracts: Cold extraction was carried out at room temperature for 48 hours by adding 50 *g* of powdered material to 250 ml ethanol. The extracts were filtered and centrifuged at 9000 g for five min. The clear supernatant obtained was passed through the membrane filter (cellulose-nitrate, 0.20 µm, Pall Gelman). The extracts were evaporated to dryness to get the residue. The weight of the residue was recorded to obtain percent extractive values.

Phytochemical Tests: were carried out using *in vivo* and *in vitro* plant material for detection of steroids, alkaloids and tannins as per the methods described by Harborne, 1998 [12]. Alkaloid extraction was carried out [12] and percentage of alkaloids was calculated for stem with bark, leaves and *in vitro* plant material.

TLC Analysis: TLC analysis of ethanolic extracts of *C. grandiflora* were loaded on precoated plates ($60F_{254}$ pre-coated (20x 20 cm Merck Darmstadt). Solvent system used for separation of compounds was cyclohexane: chloroform: diethyl amine (6:3:1) v/v. The solvent system was prepared by using AR grade solvents. The plates were run in duplicate.

For detection of alkaloids, FCPA reagent (3 % $FeCl_3$ in 35 % $HClO_4$) was used. The plates were heated in an oven till colors developed. Rf and colour of the spots were recorded.

HPLC Analysis: HPLC analysis of the ethanolic extracts of *C. grandiflora* was carried out as described in our earlier report [10]. Isocratic analytical HPLC was carried out using RPC18 column (Perkin Elmer, series 200, Switzerland, SPHERI-5, 5 mm, 250 × 4.6 mm). The mobile phase for alkaloid elution was acetonitrile : water (40:60), at a flow rate 1.6 mL/min with a sample size of 20 µL and UV detection at 254 nm. A standard curve was obtained using authentic sample of camptothecin (Sigma Aldrich). The standard was prepared using DMSO: methanol (1:50 v/v).

HPLC analysis of standard as well as extract yielded chromatogram with retention time of 3.75 min. Co-chromatography of the extracts was performed with authentic samples for confirmation. Validation of quantitative method was performed for samples in 5 replications. The results from the samples at two concentrations did not alter the retention time. The retention time proved that accuracy and reproducibility was excellent.

Antibacterial Activity: Antibacterial activity of ethanolic extracts of *C. grandiflora*, was tested against *Bacillus subtilis* (ATCC 6633) *Salmonella typhae* and *Klebsiella pneumonae*, procured from NCIM, NCL, Pune and Department of Biotechnology, Sinhagad College, Pune. Ethanolic extracts of stem with bark and leaf callus, were prepared by adding ethanol to the residues, to get the concentration 250 ppm and 500 ppm respectively. Nutrient agar medium was prepared. Bacterial cultures were inoculated into nutrient broth and incubated at 35±2°C.

After 24 hours, the bacterial suspension was centrifuged at 6000 rpm for 15 min. The pellet was suspended in sterile distilled water and the transmittance of suspension was corresponding to 70-80% at 530 nm and a constant number 10^6 cells/ml. 0.1 ml of suspension was spread on the plates. The wells were prepared by using 0.6 mm borer and 5 wells were prepared in each petridish. 75µl of extract was loaded in each well. The solvent ethanol was kept as a negative control and the standard antibiotic

Cephotaxime (250 and 500 ppm respectively) was used as a positive control. 3 replicates were kept for each set of experiment and each set was repeated twice. Inhibition zone was recorded in mm after 24 hours.

RESULTS:

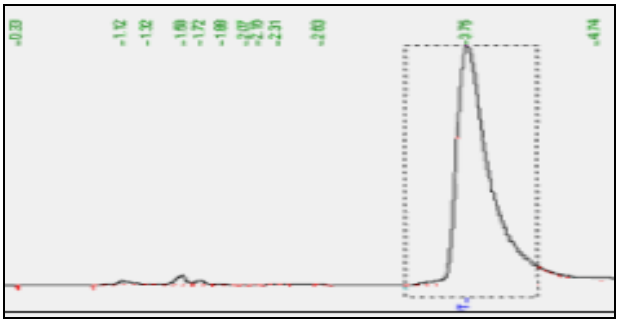
Phytochemical Investigations: revealed the presence of steroids, alkaloids and tannins in stem with bark and leaves. Presence of alkaloids was also detected in callus and *in vitro* plantlets. Percent extractive values for different plant parts of *C. grandiflora* were as recorded in **Table 1**. The callus showed higher percentage of extractive value as compared to *in vitro* plantlets. Percentage of alkaloids/ gm dry weight of plant material was the highest in stem with bark and the lowest in the *in vitro* plantlets (Table 1).

TLC analysis in *C. grandiflora:* Ethanolic extract of stem with bark , *in vitro* plantlets and callus were used to separate alkaloids on TLC plates. The results obtained were as given in table 1. It was observed that alkaloid spots in extracts of stem bark and leaves showed the same Rf. Leaf and leaf callus showed 2 and 5 alkaloid spots respectively. Variation in Rf of alkaloid spots in callus and *in vitro* plant material was observed.

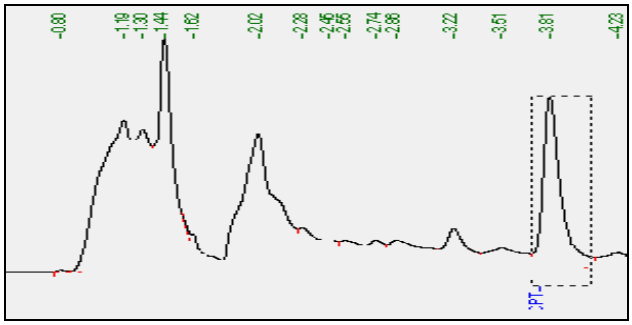
TABLE 1: PHYTOCHEMICAL ANALYSIS OF *CHONEMORPHA GRANDIFLORA*

Plant material	% extractive value (Ethanol)	% alkaloids (per gm dry weight)	Rf of alkaloid spots*
Stem with bark	6.89	0.22	0.35, 0.42, 0.56
Leaves	4.76	0.147	0.35, 0.42
Callus	3.4	0.065	0.35, 0.42, 0.62,0.72,0.8
***In vitro* plantlets**	2.3	0.047	0.35, 0.55

HPLC Analysis: In HPLC analysis of ethanolic extracts of stem with bark and leaves of *C. grandiflora,* a peak having same retention time as that of pure camptothecin was recorded (Plate 1). Thus, on the basis of retention time, we can say that camptothecin is present in these extracts of *C. grandiflora* collected from Dandeli, Karnataka state. The amount of camptothecin calculated in the stem with bark extract of *C. grandiflora* was 0.012 mg/g. It is less as compared as compared to the amount of camptothecin reported by us in ethanolic extracts of the stem with bark of *C. grandiflora* collected from Kerala state (0.013 mg/g.)[10]. The amount of camptothecin calculated for the leaves of *C. grandiflora* was 0.007 mg/g.



1



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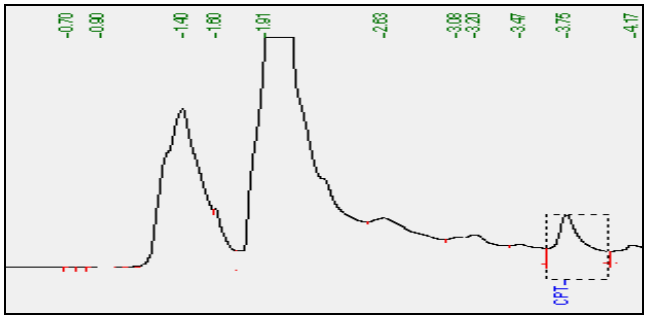

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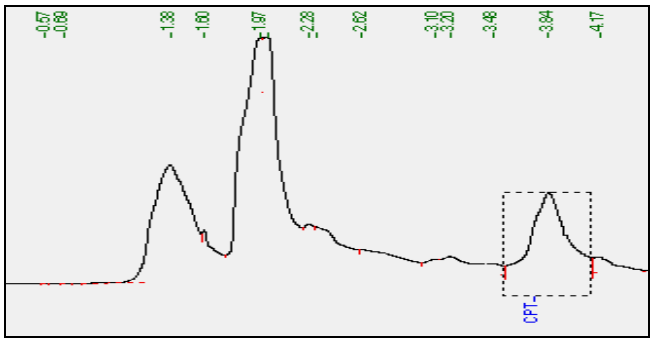

4

1. Pure sample camptothecin (40 µg /ml); in DMSO: Methanol (1:50) Retention time- 3.75 min
2. Stem with bark. Retention time-3.81 min.
3. Leaf Retention time- 3.75 min.
4. Leaf co- chromatography (10 µl sample+10µl standard). Retention time- 3.84 min

PLATE 1: HPLC ANALYSIS OF ETHANOLIC EXTRACTS OF *CHONEMORPHA GRANDIFLORA*

Antibacterial Activity: Based on phytochemical analysis, ethanolic extracts of stem with bark and leaf callus of *C. grandiflora* were selected for studies on antimicrobial activities. The stem with bark extracts showed moderate activity against *Klebsiella pneumonae and Bacillus subtilis* and no activity against *Salmonella typhae*. It is important to note that callus also showed comparable antibacterial activity to that of stem with bark extracts against *Bacillus subtilis* and *Bacillus subtilis*. Callus extracts showed maximum inhibition of *Klebsiella pneumonae* and no inhibition of *Salmonella typhae* (**Table 2**).

TABLE 2: ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACTS OF CHONEMORPHA *GRANDIFLORA*

Test organism	Diameter of inhibition zone (mm)*					
	Standard (cephotaxime)		Stem with bark		Callus	
	500 ppm	250 ppm	500 ppm	250 ppm	500 ppm	250 ppm
Bacillus subtilis	23	20	20	17	18	15
Bacillus subtilis	20	18	13	11	11	10
Salmonella typhae	24	19	11	10	--	--

*mean of 3 replicates; -- No inhibition zone

DISCUSSION: Camptothecins are one of the most important anticancer alkaloids of the 21st century because of their clinical applications against cancer [13], HIV [14], *Leishmania* [15] and *plasmodium falciparum* [16].

Camptothecin is known to occur in different unrelated genera, including *Camptotheca acuminata*, *Nothapodytes nimmoniana, Tabernaemontana heyneana* [17]. It has been recently reported in *Ophiorrhiza rugosa* var. *prostrata* [18] and in *C. grandiflora* [10]. As the demand of camptothecin is very high and supply is short, there is urgent need of finding an alternative plant source of camptothecin. So phytochemical investigations on *C. grandiflora* from different localities is needed. This could help in finding the elite plants for production of camptothecin.

The antibacterial activity of stem with bark extracts of *C. grandiflora* may be assigned to the secondary metabolites i.e., alkaloids, steroids and tannins present in the bark. The results obtained also are supported by our phytochemical investigations which have shown higher percentage of alkaloids in the bark as compared to callus. Callus showed comparable activity with that of stem bark extracts, though percentage of total alkaloids recorded in it was less. This may be due to the presence of other metabolites in callus or undifferentiated nature of callus. Further phytochemical investigations on callus are needed.

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