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New light on *Chonemorpha grandiflora*, (Roth) M.R. and S.M. Almeida, a medicinal plant for Tribal health

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Abstract

Chonemorpha grandiflora, (Roth) M.R. and S.M. Almeida syn *C. fragrans* (Moon), Alston (Apocynaceae) is medicinal plant used since ancient times. It is used in medicinal preparations such as kumaryasavam and sudarsanasavam used in Kerala Ayurvedic system. Entire plant, roots and root bark are used for fever, skin diseases , inflammations, gynaecological disorders and stomach disorders. It is also used as blood purifier by some tribes in Kerala.

Phytochemical investigations on *C. grandiflora* from Kerala, Karnataka and Maharashtra state by the author has revealed the presence of camptothecin, an important anticancer compound . Camptothecin in ethanolic extracts of stem with bark of *Chonemorpha grandiflora* was more in the plants from Kerala state(13 mg/kg)than those collected from Karnataka (12 mg/kg) and Maharashtra state (10 mg/kg). Camptothecin is an important anticancer compound useful in the treatment of colorectal cancer, lung cancer as well as blood cancer. Thus use of this plant as a blood purifier by tribes in Kerala is justified. The ethanolic extracts of stem barks of plants from Kerala locality also exhibited significantly higher anti-bacterial activity mainly against pathogenic organisms. Further investigations on this plant would help in improvement of tribal health.

Keywords: *Chonemorpha grandiflora*, Kerala Ayurvedic system, Apocynaceae, blood purifier,c amptothecin, anticancer compound

Introduction

Chonemorpha. grandiflora (Roth) M.R. and S.M Almeida syn. *C. fragrans* syn. *C. macrophylla* is a medicinal plant used in Indian medicinal systems [1, 2]. It is called Murva in Sanskrit, Chandruvoo in kannada and Perumkurumba in Kerala. It is used in medicinal preparations such as kumaryasavam and sudarsanasavum used in Kerala Kumaryasavam is used as tonic against anemia, stomach disease and mensus. 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], and the root infusion is taken for the retained placenta.

C. grandiflora, has been an exploited medicinal plant in Kerala [5]. There are very scanty reports on phytochemical analysis of 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 Karnataka state, Maharashtra and Kerala 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.
- Preparation of extracts-** Cold extraction was carried out at room temperature for 48 hours 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.
- HPLC analysis-** HPLC analysis of the ethanolic extracts of *C. grandiflora* was carried out as described in our earlier report [6]. Isocratic analytical HPLC was carried out using RPC18 column (Perkin Elmer, series 200, Switzerland, SPHERI-5, 5 mm, 250 × 4.6 mm).

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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 μ L; 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

4. **Antibacterial activity:** Antibacterial activity of ethanolic extracts of *C. grandiflora*, was tested against *E. coli*, *Bacillus subtilis* (ATCC 6633) *Salmonella typhae* and *Klebsiella pneumoniae*, procured from NCIM, NCL, Pune and Department of Biotechnology, Sinhgad College, Pune. Ethanolic extracts of stem bark were prepared by adding ethanol to the residues, to get the concentration 250 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 10⁶ 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. 75 μ l of extract was loaded in each well. The solvent ethanol was kept as a negative

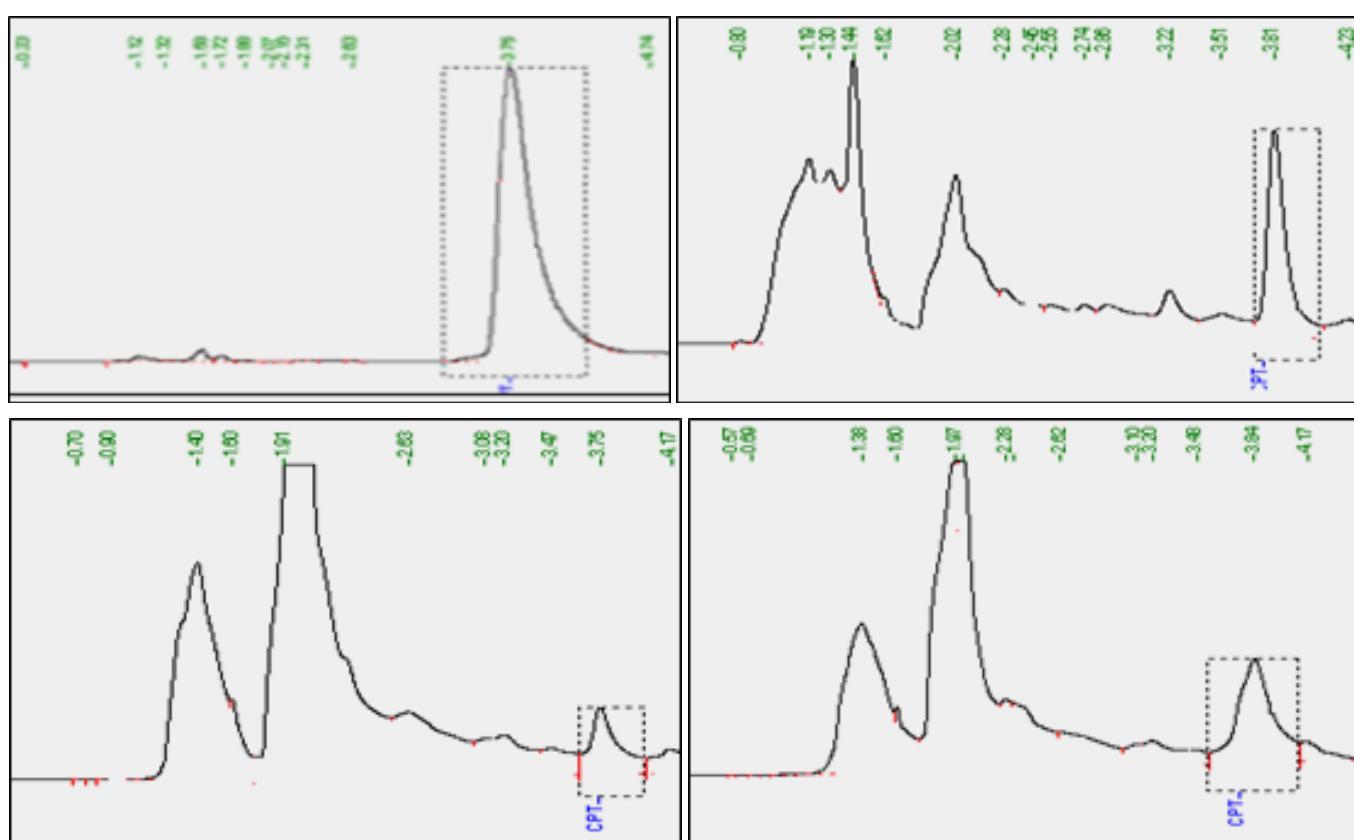
control and the standard antibiotic Cephotaxime (250 and 500 ppm respectively) was used as a positive control. 3 replicates were kept for each set of experiment and each set was repeated twice. Inhibition zone was recorded in mm after 24 hours.

Results

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). The amount of camptothecin calculated in the plant material of *C. grandiflora* from Karnataka state was 12 mg/kg. 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 (13 mg/kg). *C. grandiflora* from Maharashtra state was 10 mg/kg.

Antimicrobial activity

The stem extracts of *C. grandiflora* showed moderate activity against *Klebsiella pneumoniae*, *Salmonella typhae* and *E. coli*. Maximum inhibition was shown in case of *Bacillus subtilis*. Maximum antibacterial activity was exhibited by extracts of plants from Kerala region. It is important to note that antibacterial activity of stem extracts is exhibited against all these pathogenic organisms. (Table 1).



1. Pure sample camptothecin (40 μ g /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 μ l sample+10 μ l standard)

Plate 1: HPLC analysis of ethanolic extracts of *Chonemorpha grandiflora*

Table 1: Antibacterial activity of *Chonemorpha grandiflora* ethanolic extracts

Plant Extract	Size of inhibition zone (mm)*															
	<i>Bacillus subtilis</i>				<i>Klebsiella pneumoniae</i>				<i>Salmonella typhae</i>				<i>E.coli</i>			
	Standard		Extract		Standard		Extract		Standard		Extract		Standard	extract		
	500 ppm	250 ppm	500 ppm	250 ppm	500 ppm	250 ppm	500 ppm	250 ppm	500 ppm	250 ppm	500 ppm	250 ppm	500 ppm	250 ppm	500 ppm	250 ppm
	50	25			30	20			20	15			35	20		
Stem bark Kerala		35	22			20	8			15	10			25	17	
Stem bark (Karnataka)		25	20			15	8			11	7			20	15	
Stem bark Maharashtra		18	8			12	8			10	7			16	6	

*Inhibition zone in mm Mean of 3 replicates

Discussion

Camptothecins are one of the most important anticancer alkaloids of the 21st century because of their clinical applications against cancer.⁷ colorectal cancer, lung cancer as well as blood cancer. As we have reported presence of camptothecin in *C. grandiflora*, use of this plant as a blood purifier by tribes in Kerala is justified. The antibacterial activity of extracts of plants from Kerala region also is comparable with standard antibiotic cephalexine. The plant extracts are used for curing inflammation by the tribes in Kerala is also justified. The antibacterial activity was maximum for Kerala plant extracts which may be due to Camptothecin and other secondary metabolites. Further such investigations on this plant and the other medicinal plants in ancient literature is needed. This would help in the development of herbal drugs in improvement of tribal health.

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