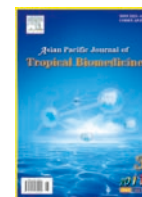




Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading doi:10.1016/S2221-1691(11)60022-1 © 2011 by the Asian Pacific Journal of Tropical Biomedicine. All rights reserved.

Pharmacognostic study and anti-inflammatory activity of *Callistemon lanceolatus* leafKumar S^{*}, Kumar V, Prakash OM

Institute of Pharmaceutical Sciences, Kurukshetra University, Haryana, India

ARTICLE INFO

Article history:

Received 10 February 2011

Received in revised form 3 March 2011

Accepted 15 March 2011

Available online 30 March 2011

Keywords:

Anti-inflammatory

Callistemon lanceolatus

Carrageenan

Fibre

Microscopy

Stomata

ABSTRACT

Objective: To study detail pharmacognosy and anti-inflammatory activity of *Callistemon lanceolatus* (*C. lanceolatus*) leaf. **Methods:** Leaf sample was studied by organoleptic, macroscopical, microscopical, phytochemical and other WHO recommended methods for standardizations. The methanolic leaf extract of the plant was also screened for anti-inflammatory activity on carrageenan-induced paw edema in rat at doses of 200 and 400 mg/kg, orally. The detail pharmacognostic study of the *C. lanceolatus* leaf was carried out to lay down the standards which could be useful in future experimental studies. **Results:** *C. lanceolatus* methanolic leaf extract showed significant ($P < 0.05$) anti-inflammatory activity at doses of 200 mg/kg and 400 mg/kg. This significant anti-inflammatory of *C. lanceolatus* methanolic leaf extract at the dose of 400 mg/kg was comparable with diclofenac sodium. **Conclusions:** The pharmacognostic profile of the *C. lanceolatus* leaf is helpful in standardization for quality, purity and sample identification. The methanolic extract at a dose of 400 mg/kg shows a significant activity in comparison with the standard drug diclofenac sodium (50 mg/kg).

1. Introduction

Callistemon lanceolatus (*C. lanceolatus*) (Family: Myrtaceae) commonly known as bottle brush, is frequently cultivated throughout India in gardens as ornamental plant^[1,2]. Aqueous extracts of the leaves and flowers have antifungal and antibacterial activity. The plant extracts inhibited urd bean leaf crinkle virus *in vitro* studies. The extract also shows cholinesterase activity^[3,4]. The ethanol extract of *C. lanceolatus* showed strong elastase inhibition and DPPH radical scavenging activities^[5]. The essential oils from leaves possess antimicrobial, fungitoxic, antinociceptive and anti-inflammatory activities. Several triterpenoids, flavonoids, fatty acids, tannins, and phenolic compounds have been isolated from its leaves^[6,7]. The present work was undertaken to study pharmacognostic characteristics and anti-inflammatory activity of *C. lanceolatus*.

2. Material and methods

2.1. Chemicals

Carrageenan was purchased from s d-fine Chem. Limited, Mumbai, India. Phloroglucinol, glycerin, hydrochloric acid, chloral hydrate, potassium hydroxide and all other chemicals used in the study were of analytical grade.

2.2. Plant material

C. lanceolatus leaves were collected from the campus of Kurukshetra University, Kurukshetra, India and were identified by Dr Singh HB, Scientist F & Head, Raw Material Herbarium & Museum, NISCAIR, New Delhi, India. A voucher specimen of the plant was preserved in the herbarium (NISCAIR/RHMD/Consult/-2009-10/1381/182/2).

2.3. Extract preparation

The leaves were washed with water and shade-dried. The dried leaves were powdered by using dry grinder and passed through sieve (60 #). Previously defatted powder material with petroleum ether [(60–80) °C], was packed into Soxhlet apparatus and extracted with methanol. The

*Corresponding author: Kumar S, Assistant Professor, Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra-136119, Haryana, India.

Tel: + 91-9416581005, +91-1744-239617

Fax: +91-1744-238277

E-mail: sunilmadhuban@yahoo.com

Foundation Project: Supported by a grant from AICTE, New Delhi (No. 1-51/RID/CA/4/2009-10).

extract was evaporated to dryness under reduced pressure at 45 °C to give solid residues. The residue was stored in airtight containers in refrigerator below 10 °C for subsequent experiments.

2.4. Animals

Wistar rats of either sex, weighing about 150–250 g were used in the study. Animals were maintained under standard environmental conditions *i.e.* ambient temperature of (22 ± 2) °C and at 45%–55% relative humidity for 12 h, each of dark and light cycle and fed with a standard pellet rats diet obtained from Ashirwad Industries, Chandigarh, India and water was supplied *ad libitum*.

2.5. Pharmacognostic study

Fresh leaves were taken for morphological and histological studies. Coarse powder (60 #) was used to study microscopical characters of leaf powder, physiochemical parameters and phytochemical investigation. The detailed pharmacognostic studies of the plant leaf were carried out according to well known methods and procedures^[8–12].

2.6. Anti-inflammatory activity

The carrageenan-induced rat paw edema test was performed according to the method described by Winter *et al*^[13]. The control group received simple saline (group I), the standard group (group II) received diclofenac sodium (50 mg/kg), *i.p.* and the test groups (group III & IV) received extract at the doses of 200 and 400 mg/kg administered orally. 30 min after administration of extract, 0.1 mL of 1% w/v of carrageenan suspension was injected to all animals in the left hind paw (plantar region). The paw volume, up to the tibiotarsal articulation, was measured using a plethysmometer (model 7140, Ugo Basile, Italy). The measures were determined at 1, 2 and 3 h after drug treatment.

2.7. Statistical analysis

Statistical analysis was done using one way analysis of variance followed by Dunnett's test. *P* values less than 0.05 were considered as significant.

3. Results

3.1. Pharmacognostic study

3.1.1. Macroscopic characteristics

C. lanceolatus is shrub or small tree growing upto 7.5 m in height (Figure 1a). The leaves of the plant are lanceolate (Figure 1b) with prominent veins, midrib and oil glands (0.25–0.7 cm wide and 3.5–7.6 cm long). The organoleptic evaluation of the leaf powder revealed that leaf powder is pale green in color, with a characteristics odour and taste.

3.1.2. Microscopical characteristics

3.1.2.1. Leaf microscopy

The leaf surface shows the anomocytic types of stomata which is characteristics of family Myrtaceae (Figure 2a). Leaf surface analysis also shows the presence of veins, vein-islets and vein terminations (Figure 2b). Transverse section of leaf (Figure 3a) shows the epidermis layer followed by cuticle layer, and vascular bundles (xylem and phloem), pericyclic fibres, collenchymas, *etc.* The vascular bundles and pericyclic fibers were stained pink with phloroglucinol and HCl (Figure 3b). The mesophyll is differentiated into palisade and spongy parenchyma. Many covering trichomes emerge from the upper epidermis. Trichomes are unicellular. Strips of collenchyma are present below and upper layer of epidermis. Leaf constants such as stomatal number, stomatal index, veinlet terminations and vein-islet number were measured. The results were shown in Table 1.

Table 1
Leaf constants.

Parameters	Value (in 1 mm ² area)
Average stomatal number in 25 different fields (100×)	83.0
Average stomatal number in 25 different fields (400×)	90.0
Stomatal index (100×)	8.7
Stomatal index (400×)	9.0
Vein-islet number (20×)	4.0–6.0
Vein-termination number (20×)	3.0–5.0

×= magnification power

3.1.2.2. Powder microscopy

The fine powder was mounted in glycerin as well as stained (phloroglucinol + HCl). Observation of microscope showed presence of unicellular trichomes, anomocytic stomata, calcium oxalate crystals, epidermal cells, xylem vessels (Figure 4), pericyclic fibers, *etc.*

3.1.3. Preliminary phytochemical screening

Preliminary phytochemical screening mainly revealed the presence of steroids, terpenoids, saponins, fatty acids, flavonoids, phenolic compounds, alkaloids and carbohydrates.

3.1.4. Physiochemical parameters

Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The percentage of total ash was 4.48% w/w, acid insoluble ash was 2.55% w/w, water soluble ash was 0.55% w/w, foreign matter was 0.41% w/w, loss on drying was 3.50% w/w and swelling index was 0.65 mL. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. Pet. ether soluble extractive was 8.2% w/w, chloroform soluble extractive was 7.5% w/w, methanol soluble extractive was 35.8% w/w, water soluble extractive was 25.4% w/w and alcohol soluble extractive was 27.3% w/w.

3.2. Anti-inflammatory activity

Table 2
Anti-inflammatory activity of methanolic leaf extract (mean±SEM).

Groups	Paw volume (mL)		
	1 h	2 h	3 h
Group I: Control	1.761±0.185	2.370±0.127	2.157±0.183
Group II: Diclofenac sodium (50 mg/kg)	1.602±0.221	1.237±0.127*	1.140±0.268 **
Group III: CLME (200 mg/kg)	1.638±0.138	1.491±0.143*	1.412±0.152*
Group IV: CLME (400 mg/kg)	1.638±0.183	1.323±0.143*	1.270±0.207**

** $P < 0.001$; * $P < 0.05$.



Figure 1. a: *C. lanceolatus*; b: Lanceolate leaf showing tichomes on surface.

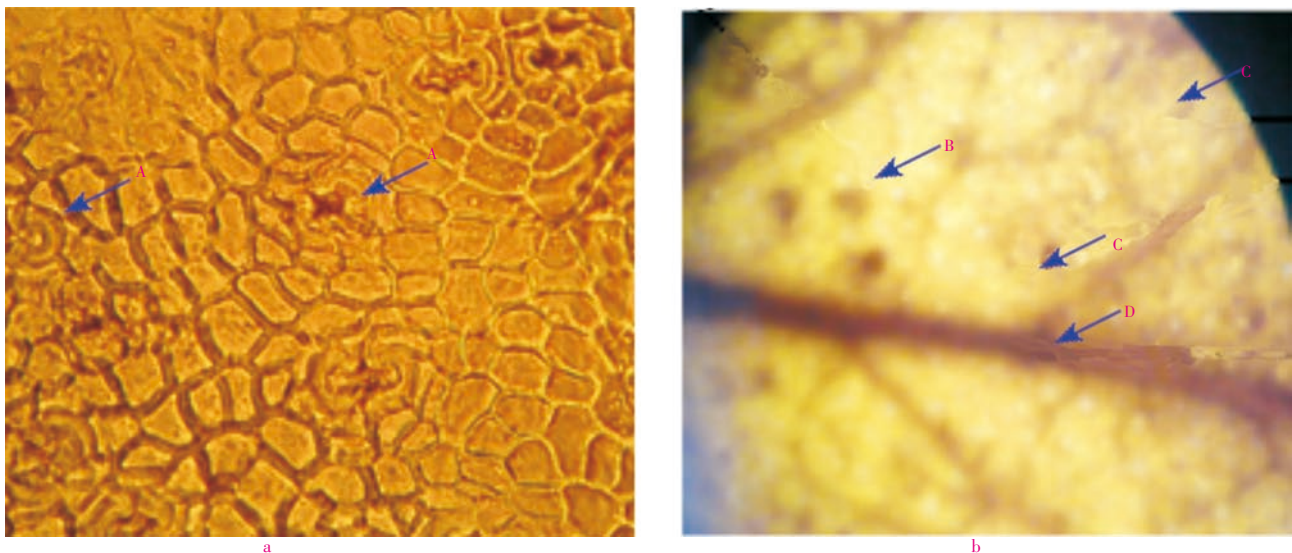


Figure 2. Leaf surface (a: Stomata; b: Veins, veinlet termination, vein-islet & midrib).
A: Anomocytic stomata; B: Veinlet termination; C: Vein-islet; D: Midrib.

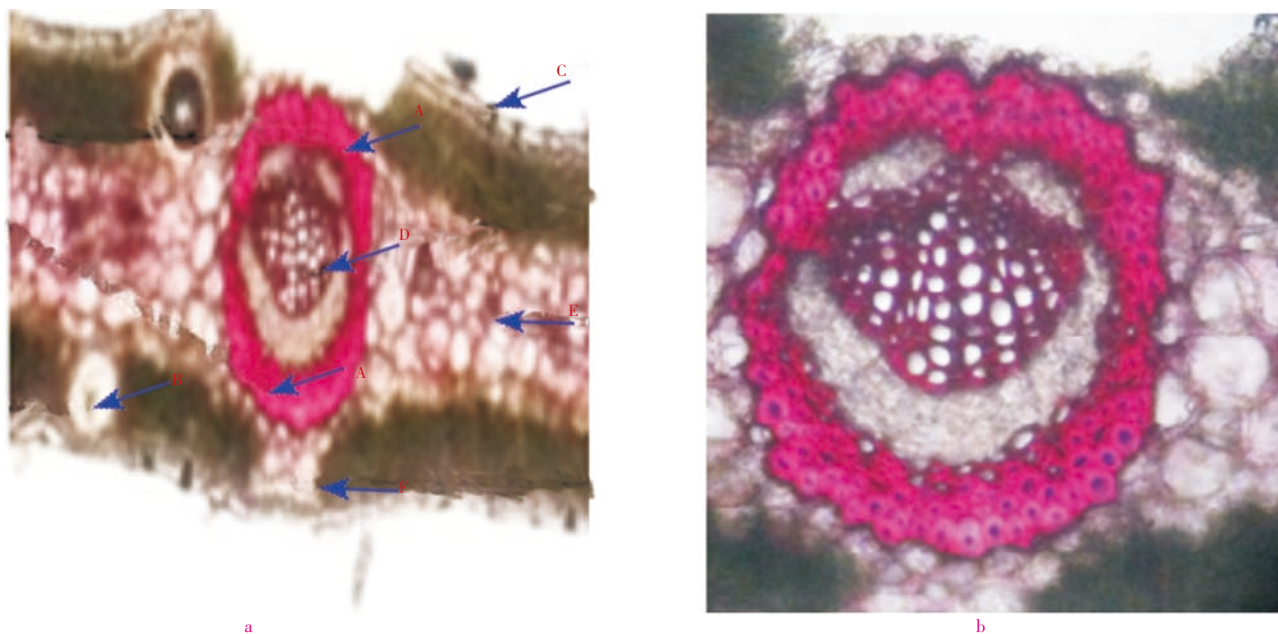


Figure 3. a: T.S. of *C. lanceolatus* leaf (100 X); b:Vascular bundles & pericyclic fibres (400X).
 A: Pericyclic fibres; B: Oil glands; C: Epedermis; D: Vascular bundles; E: Spongy parenchyma; F: Collenchyma.

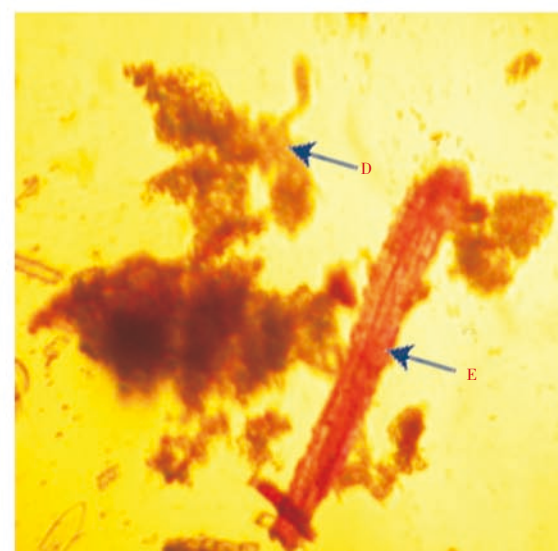
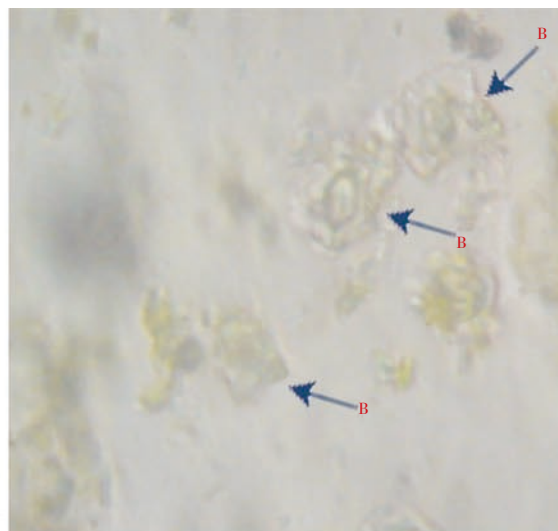
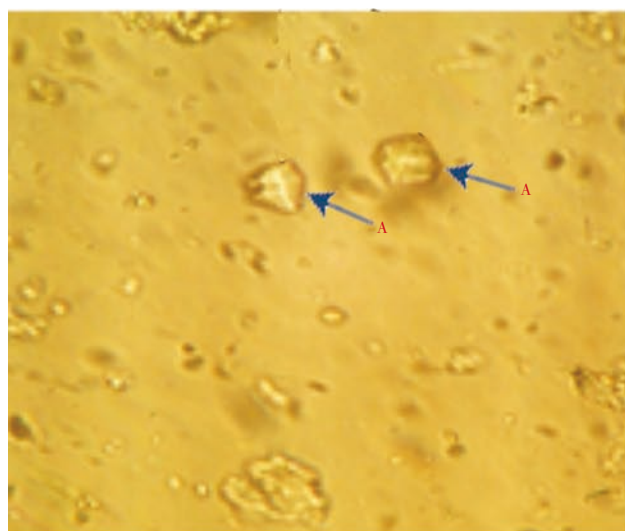


Figure 4. Powder characteristics of *C. lanceolatus* leaf (400X).
 A: Ca-oxalate crystals; B: Anomocytic stomata; C: Trichome; D: Parenchymatous cells; E: Xylemvessel.

C. lanceolatus methanolic leaf extracts (200 and 400 mg/kg) significantly inhibited carrageenan-induced rat paw oedema formation (Table 2). The inhibition of oedema by extract was dose dependent. The anti-inflammatory activity was compared with diclofenac sodium (50 mg/kg). The activity showed by *C. lanceolatus* methanolic leaf extract (400 mg/kg) was comparable to diclofenac sodium after 3 h of experiment.

4. Discussion

Standardization is essential measure for quality, purity and sample identification. Microscopic method is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials^[14]. The pharmacognostic standards for the leaves of *C. lanceolatus* are carried out for the first time in this study. Morphological and histological studies of the leaf will enable to identify the crude drug. The information obtained from preliminary phytochemical screening will be useful in finding out the genuity of the drug. Ash values, extractive values can be used as reliable aid for detecting adulteration. These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy^[15]. The macroscopical characters of the leaf can serve as diagnostic parameters. The microscopical studies of the transverse section showed presence of unicellular trichomes and anomocytic stomata, which are characteristics of the family Myrtaceae. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent^[16]. Preliminary phytochemical analysis indicated the presence of steroids, terpenoids, alkaloids, fatty acids, flavonoids, phenolic compounds and carbohydrates. The methanolic extract was also screened for anti-inflammatory activity.

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury^[17]. Carrageenan induced inflammation is a useful model for the estimation of anti-inflammatory effect^[18]. The development of oedema in the paw of the rat after the injection of carrageenan is due to the release of histamine, serotonin, prostaglandin and the like^[17,19,20]. *C. lanceolatus* methanolic leaf extract showed significant ($P < 0.05$) anti-inflammatory activity at doses of 200 and 400 mg/kg. This significant anti-inflammatory of *C. lanceolatus* methanolic leaf extract at the dose of 400 mg/kg was comparable with diclofenac sodium. The presence of bioactive constituents as indicated above may be responsible for anti-inflammatory activity. However, the main active constituents responsible for the activity should be isolated from the plant.

In conclusion, pharmacognostic parameters could be useful to detect the authenticity of this medicinally useful plant. Furthermore, the methanolic extract of the leaves have potent anti-inflammatory activity.

Conflict of interest statement

We declare that we have no conflict of interest.

References

- Sharma RK, Kotoky R, Bhattacharyya PR. Volatile oil from the leaves of *Callistemon lanceolatus* D.C. grown in north-eastern India. *Flavour Fragr J* 2006; **21**: 239–240.
- Anonymous. The wealth of India raw material. New Delhi: Council of Scientific & Industrial Research; 1992, p. 63–65.
- Chowdhary AK, Saha NK. Inhibition of urd bean leaf crinkle virus by different plant extracts. *Indian Phytopathology* 1985; **38**: 566–568.
- Gupta A, Gupta R. A survey of plants for anticolinestrace activity. *Phytochemistry* 1997; **46**: 827–831.
- Kim JH, Byun JC, Reddy BAK, Chang-Gu H, Lee NH. Compounds with elastase inhibition and free radical scavenging activities from *Callistemon lanceolatus*. *J Med Plant Res* 2009; **3**(11): 914–920.
- Sudhankar M, Rao CV, Rao A L, Raju DB. Antinociceptive and anti-inflammatory effects of the standardized oil of Indian *Callistemon lanceolatus* leaves in experimental animals. *Acta Pharm Turc* 2004; **46**: 131–139.
- Jeong W, Su HS, Kim N, Yang YT, Su SY, Lee C, et al. Bioactive triterpenoids from *Callistemon lanceolatus*. *Arch Pharm Res* 2009; **32**(6): 845–849.
- Brain KR, Turner TD. *The practical evaluation of phytopharmaceuticals*. Bristol: Wright-Scientifica; 1975, p. 4–9.
- Ministry of Health and Welfare. *Indian pharmacopeia*. 4th ed. New Delhi: Government of India, Ministry of Health and Welfare, Controller of publications; 1996, p. A53–A54.
- Khandelwal KR. *Practical pharmacognosy*. 18th ed. Pune: Nirali Publication; 2007.
- Kokate CK. *Practical pharmacognosy*. 1st ed. New Delhi: Vallabh Prakashan; 1986.
- WHO. *WHO/PHARMA/92.559/rev.1. Quality control methods for medicinal plant material*. Geneva: Organisation Mondiale De La Sante; 1992, p. 22–34.
- Winter CA, Risley EA, Nuss GW. Carrageenan induced oedema in hind paws of the rats as an assay for anti-inflammatory drugs. *Proc Soc Exp Biol Med* 1962; **111**: 544–557.
- Singh S, Machawal L, Chauhan MG. Pharmacognostic study of male leaves of *Trichosanthes dioica* Roxb. with special emphasis on microscopic technique. *J Pharmacogn Phytother* 2010; **2**(5): 71–75.
- Nayak BS, Patel KN. Pharmacognostic studies of the *Jatropha curcas* leaves. *Int J Pharm Tech Res* 2010; **2**(1): 140–143.
- Thomas S, Patil DA, Patil AG, Chandra N. Pharmacognostic evaluation and physicochemical analysis of *Averrhoa carambola* L. fruit. *J Herbal Med Toxicol* 2008; **2**(2): 51–54.
- Mohamed STK, Azeem AK, Dilip C, Sankar C, Prasanth NV, Duraisami R. Anti-inflammatory activity of the leaf extracts of *Gendarussa vulgaris* Nees. *Asian Pac J Trop Biomed* 2011; **1**(2): 147–149.
- Ratheesh M, Helen A. Anti-inflammatory activity of *Ruta graveolens* Linn on carrageenan induced paw edema in wistar male rats. *Afr J Biotechnol* 2007; **6**(10): 1209–1211.
- Georgewill OA, Georgewill UO, Nwankwoala RNP. Antiinflammatory effects of *Moringa oleifera* lam extract in rats. *Asian Pac J Trop Med* 2010; **3**(2): 133–135.
- Georgewill OA, Georgewill UO. Evaluation of the anti-inflammatory activity of extract of *Vernonia amygdalina*. *Asian Pac J Trop Med* 2010; **3**(2): 150–151.