Natural Product Sciences
14(4) : 221-226 (2008)

Pharmacognostical Evaluation of the Bark of *Careya arborea* (Roxb.)

R Vijaya Bharathi*, K. Rajendran[1], Mustafa Mohamed Ali Saeed[2], Rubina Kaur[2], SN Soundarya Lakshmi[2], and RK Vasanthi[2]

**Department of Pharmacognosy, Madras Medical College, Park Town,Chennai-600 003*
[1]Department of Pharmacy, 7th April University, Al-zawia, Libya
[2]Department of Pharmacognosy, C.L. Baid Metha College of Pharmacy, Chennai-600 097, Tamil Nadu, India.

Abstract – The bark of *Careya arborea* Roxb. (Barringtoniacae) is used against various disorders in Indian systems of medicine, namely astringent, anthelmintic, in tumors, dyspepsia, colic, bronchitis, urinary discharges, piles, lecucoderma, skin diseases, epileptic fits, diarrhoea. The juice of the fresh bark with honey is given as a demulcent in coughs and colds, infusion of the fresh bark is used to treat snake bite by applying to the bitten part and also taken internally. The present communication deals with the detailed pharmacognostical evaluation of the bark sample using light and confocal microscopy, WHO recommended physico-chemical determinations and authentic phytochemical procedures. The physico-chemical, morphological and histological parameters presented in this paper may be proposed as parameters to establish the authenticity of *C. arborea* bark and may possibly help to differentiate the drug from its other allied species.
Keywords – *Careya arborea*, Barringtoniacae, bark, pharmacognosy, HPTLC.

Introduction

Careya arborea Roxb. (Barringtoniacae) is a medium sized deciduous tree and its bark used in Indian system of medicine against various disorders namely astringent, anthelmintic, in tumors, dyspepsia, colic, bronchitis, urinary discharges, piles, lecucoderma, skin diseases, epileptic fits, diarrhoea. The juice of the fresh bark with honey is given as a demulcent in coughs and colds, infusion of the fresh bark is used to treat snake bite by applying to the bitten part and also taken internally (Singh and Abrar Khan, 1990). In view of its diverse medicinal applications and in order to ensure the quality of its supply, especially in cases of adulteration and substitution prevailing on the crude drug markets, the present investigation deals with the pharmacognostical evaluation of the barks of *C. arborea.* The study includes morphological and anatomical evaluation, determination of physico-chemical constants and the preliminary phyto-chemical screening of the different extracts of *C. arborea*.

Experimental

Plant material – The barks of *C. arborea* were collected from Thirunelveli, Tamil Nadu, India during the month of October, 2007. The botanical identity of the plant was confirmed by Dr. P. Jayaraman, Botanist, Medicinal Plant Research Unit, Chennai, Tamil Nadu. A voucher specimen (PP 74) has been deposited at the Museum of the Department of Pharmacognosy, C.L. Baid Metha College of Pharmacy, Chennai.

Chemicals and instruments – Compound microscope, glass slides, cover slips, watch glass and other common glass ware were the basic apparatus and instruments used for the study. Microphotographs were taken using a Leica DMLS microscope attached with Leitz MPS 32 camera. Solvents *viz.* ethanol (95%) and reagents *viz.* phloroglucinol, glycerin, HCl, chloral hydrate and sodium hydroxide were procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India.

Macroscopic and microscopic analysis – The macroscopy and microscopy of the bark were studied according to the method of Brain and Turner (1975a). For the microscopical studies, cross sections were prepared and stained as per the procedure of Johansen (1940). The micropowder analysis was done according to the method of Brain and Turner (1975b) and Kokate (1986a).

Physico-chemical analysis – Physico-chemical analysis i.e. percentage of ash values and extractive values were performed according to the official methods prescribed

*Author for correspondence
Tel: +91-44-24960151; E-mail: rvbharathi2003@yahoo.com

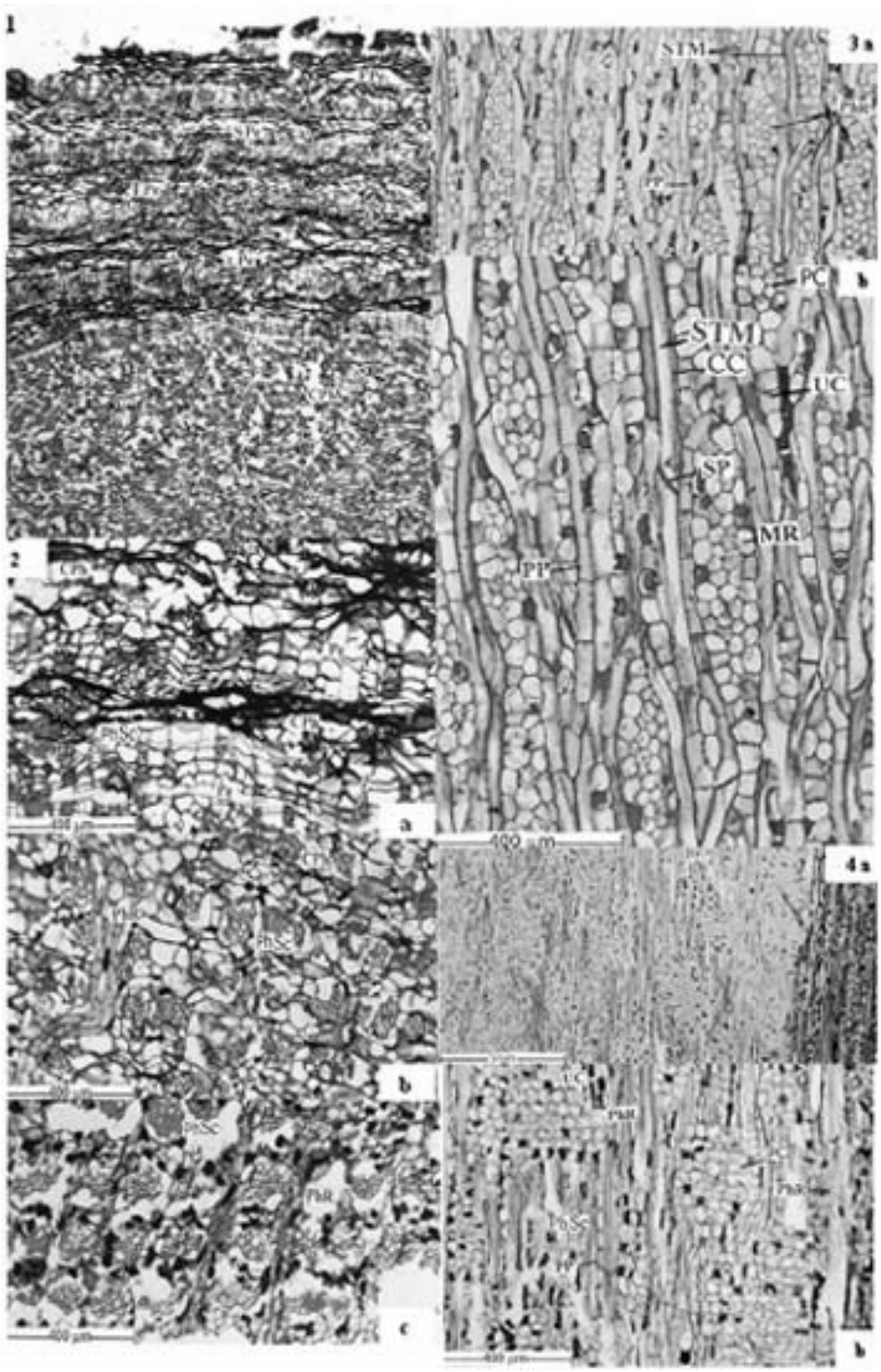

Fig. 1. T.S. of bark: (**Cph** - collapsed phloem; **Fpe** - First formed periderm; **Lpe** - Last formed periderm; **Spe** - Second formed periderm; **Tpe** - Third formed periderm).

Fig. 2. Structure of three zones of bark: **a)** Outer rhytidome **b)** Middle collapsed phloem zone **c)** Inner non-collapsed phloem zone (**Cph** - Collapsed phloem; **Pe** - Periderm; **PhR** - Phloem ray; **phsc** - Phloem sclereids).

Fig. 3. TLS view of phloem: **a**) Non-storied multi seriate wide rays **b**) Sieve tube members and sieve plate structure of phloem rays (**Cc** - Campanion cells; **MR** - Multiseriate ray; **PC** - procumbent cells; **PhR** - phloem ray; **PP** - Phloem parenchyma; **Sp** - Sieve plate; **STM** - Sieve tube member; **UC** - Upright cells).

Fig. 4. RLS of phloem: **a**) Phloem rays as seen under low magnification **b**) Same as above under high magnification (**Pc** - Procumbent cells; **PhR** - phloem ray; **PP** - Phloem parenchyma; **Phsc** - Phloem sclereids; **UC** - Upright cells).

(Indian Pharmacopoeia, 1966) and the WHO guidelines on quality control methods for medicinal plant materials (WHO/QCMMPM guidelines (1992). Fluorescence analysis was carried out according to the method of Chase and Pratt (1949) and Kokoski *et al.* (1958).

Preliminary phytochemical screening – Preliminary phytochemical screening was carried out by using standard procedures described by Kokate (1986b) and Harborne (1998).

HPTLC study – The presence of Gallic acid in EtOH extract was confirmed by co-chromatography with an authentic sample (Sigma Chemical Company, USA). Quantification of gallic acid was carried out for the EtOH extract of the bark. The linearity of the HPTLC method was investigated for lupeol in the range of 100 - 1000 µg/mL at five concentration levels using the Camag Linomat V applicator onto the precoated silica gel plate (Merck). The plate was then eluted with toluene-ethyl acetate-formic acid (5 : 5 : 1). After elution the plate was scanned densitometrically using Camag TLC scanner 3 at 278 nm. The percentage of gallic acid in the EtOH extract was calculated by calibration using peak height and peak area ratio.

Results and Discussion

Macroscopic characters of the plant – The outer surface of bark is dull to dark brown in colour and exhibits patches of greyish colored cork and exfoliating thin strips. It possesses longitudinal wrinkles. The inner surface is pale brown in colour. The bark exhibits fibrous fracture with astringent taste and measures 15 - 20 cm long and 0.5 - 1 cm in thickness.

Microscopic characters of bark

Transverse sectional view – The bark exhibits three zones, *viz.*, outer, middle and inner zones. *Outer Zone* - It is 1mm wide and consists of five or more thin successive layers of periderm and crushed phloem tissue. Periderm and crushed phloem zones alternate in successive series forming a structure termed rhytidome. The periderm layer consists of thin walled, suberised tabular cells. Each periderm zone has four to eight layers of phellem cells. Phelloderm is not evident. The phloem zones that consist of crushed cells which form tangential, dark lines of network and small masses of phloem tissues (Fig. 1).

Middle zone- It is the wider part of the bark and consists of largely crushed phloem cells, dilated phloem rays, phloem, parenchyma and abundance of phloem fibres. The crushed phloem is seen in the form of dark tangential wavy lines. Phloem parenchyma cells are dilated and are seen in large masses. Phloem rays are widen, they are either straight or wavy. The ray cells contain starch grains and prism type of calcium oxalate crystals. Steroids are also abundant in bark.

Inner zone- It is a narrow zone of phloem elements. The phloem rays are narrow and straight. No crystals, starch grains are seen in inner zone. The sieve elements and their companion cells are visible in this zone. So, the inner zone of phloem is the conducting part of the bark.

Tangential longitudinal view – The phloem shows the structure and organization of the phloem rays, the structure of sieve tubes and axial parenchyma. The phloem rays are wide and high; they are 4 to 6 seriate; 6 seriate rays are more common. The phloem rays are hetero-cellular with polygonal body cells and vertically elongated, triangular marginal cells. The rays range in height from 400 µm to 700 µm; they are 40 - 80 µm in breadth. Ray frequency is 10 to 12 / mm. Sieve tube members are straight or slightly curved. They are narrow and canal like. The sieve plate is wide and simple; it is oblique. The companion cells are narrow, vertically oblong and occur in the middle part of the sieve tube. Axial parenchyma cells are vertically rectangular; they occur in vertical strands. The cells are 100 µm long and 25 µm wide. The cells are thin walled.

Radial longitudinal view – The phloem rays are horizontally oriented in the form of wide bands. The phloem sclerenchyma are in several, parallel vertical lines. The rays show central band of procumbent cells which are horizontally elongated or squarish in shape. The cells along the upper and lower ends are vertically elongated and are called upright cells. The axial parenchyma cells are in vertical strands as in TLS view. The sieve tube members are also in longitudinal rows.

Powder characters of bark (Fig. 5) – The bark powder is light brown in colour with a characteristic odour and slightly bitter and astringent taste. Thick walled brownish cork cells are seen. Tannins are abundant in the rhytidome, middle crushed phloem and in the inner phloem. It is dark green and occurs in amorphous dense bodies in the cells. Because of the presense of tannin, the bark appears dark in colour. Tannin is also seen in the phloem rays and parenchyma. Starch grains occur as simple, small circular with concentric hilum, abundant in the phloem ray cells and measuring 5 - 10 µm in diameter. Under polarized light microscope the starch grains appear bright with different shapes of dark masses. Calcium oxalate crystals are predominantly prismatic type and

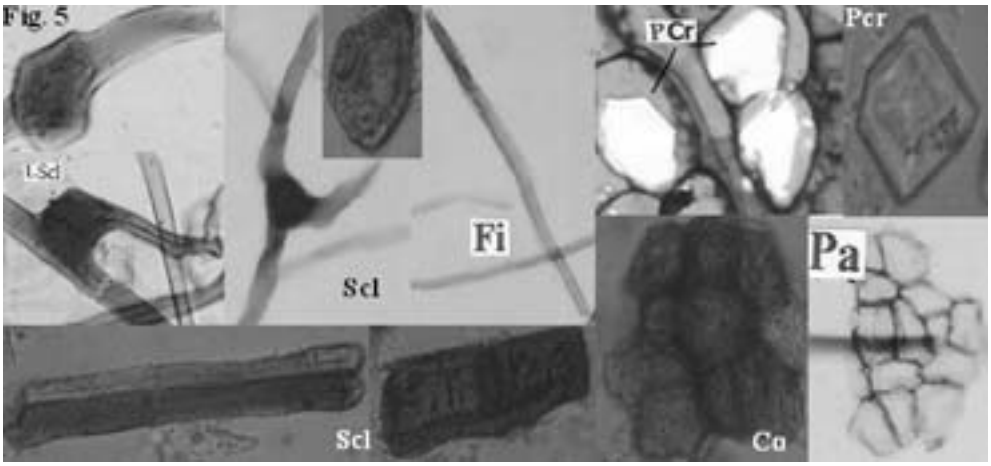

Fig. 5. Powder microscopy of the bark: **(Fi** - Fibres; **Pa** - Parenchyma cells; **Scl** - Different types of sclereids; **L Scl** - Lobed Sclereid; **Pcr** - Prism crystals; **Co** - Cork).

Table 1. Preliminary phytochemical screening of the bark powder of *C. arborea*

Test	Hexane	Benzene	Chloroform	Acetone	Ethanol	Water
Alkaloids	+	–	–	–	–	–
Carbohydrates	–	–	–	–	+	+
Phytosterols	+	+	–	–	–	–
Terpenes	+	+	–	–	–	–
Fixed oils and fats	–	–	–	–	–	–
Saponins	–	–	–	–	+	+
Phenolic compounds and tannins	–	–	–	+	+	+
Flavanoids	–	–	–	–	–	–

+ Denotes the presence of the respective class of compounds

Table 2. Ash values of the bark powder of *C. arborea*

Parameters	Values % (w/w)
Total ash	7.60
Acid insoluble ash	0.86
Water soluble ash	1.24
Sulphated ash	8.51

Table 3. Extractive values of the bark powder of *C. arborea*

Parameters	Values % (w/w)
a) Water soluble extractive	10.56
b) Ethanol soluble extractive	18.96
c) Ether soluble extractive	15.04

rhomboidal, polyhedral and cuboidal in shape. The crystals are seen mostly in the phloem ray cells. They are also seen near the fibre or sclerenchyma masses and measuring 40- 50µm long. Sclereids are long, narrow and fibres like structure. The sclereids are simple or lobed. They have thick walls, narrow lumen and canal like simple pits. Some of the sclerenchyma elements are fibres, they are similar to the sclereids but have thin walls and wider lumen.

Preliminary phytochemical screening – Preliminary phytochemical screening revealed the presence of phytosterols, lipids, phenolic compounds, carbohydrates, flavonoids and tannins (Table 1).

Physico-chemical constants – Ash value of a drug gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The ash values (Table 2) of the powdered *C. arborea* bark revealed a high concentration of sulphated ash. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The ethanol soluble extractive (Table 3) was high in *C. arborea.* The results of fluorescence analysis of the drug powder are presented in Table 4.

HPTLC study – A densitometric HPTLC analysis was performed for the quantification of gallic acid present in the EtOH extract of *C. arborea* bark. The amount of gallic acid present (R_f - 0.39) in the bark drug was found to be 0.244% w/w, and it can be used as identifying marker of this plant (Fig. 6).

Table 4. Fluorescence analysis of the bark powder of *C. arborea*

Treatment	Day light	UV light (254 nm)
Powder as such	Light brown	Dark brown
Powder + 1N NaOH (Aqueous)	Light blackish brown	Slightly greenish brown
Powder + 1N NaOH (Alcoholic)	Blackish brown	Greenish brown
Powder + 1N HCl	Brown	Blackish green
Powder + Ammonia	Reddish brown	Fluorescent greenish brown
Powder + Iodine	Reddish brown	Fluorescent greenish black
Powder + $FeCl_3$	Slightly greenish brown	Dark greenish brown
Powder + 1N H_2SO_4	Brown	Greenish brown
Powder + Acetic acid	Light brown	Greenish brown
Powder + 1N HNO_3	Slightly yellowish brown	Fluorescent greenish black

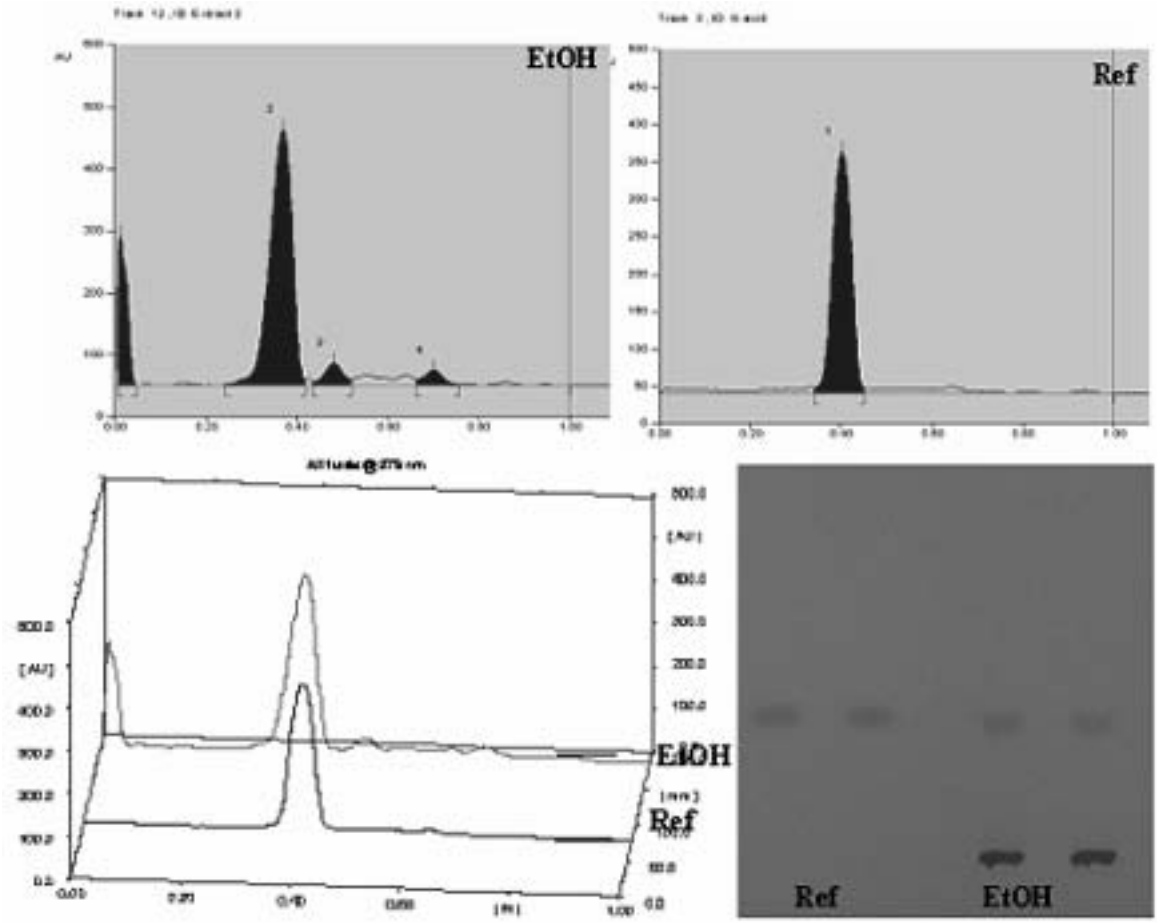

Fig. 6. HPTLC profile of EtOH extract of C. *arborea* bark and gallic acid reference: **(EtOH** - Ethanol extract; **Ref** - Gallic acid reference).

Conclusion

As there is no pharmacognostic/anatomical work on record of this traditionally much valued drug, the present work was taken up with a view to lay down standards which will contribute significantly to quality control and authentication of this medicinally useful plant *Careya arborea*.

References

Brain, K.R. and Turner, T.D., *The Practical Evaluation of Phytopharmaceuticals*, Wright-Scientechnica, Bristol, pp. 4-9 (1975a).

Brain, K.R. and Turner, T.D., *The Practical Evaluation of Phytopharmaceuticals*, Wright-Scientechnica, Bristol, pp. 36-45 (1975b).

Chase, C.R. and Pratt, R.J., Fluorescence of powdered vegetable drugs

with particular reference to development of a system of identification, *J. Am. Pharmacol. Assoc* **38**, 32 (1949).

Harborne, J.B., Methods of extraction and isolation. In: *Phytochemical Methods*, Chapman & Hall, London, pp. 60-66 (1998).

Indian Pharmacopoeia, 4th edn.,Vol. II, Government of India, Ministry of Health and Welfare, Controller of Publications, New Delhi, pp. A53-A54 (1996).

Johansen, D.A., *Plant Microtechnique*, McGraw Hill, New York, pp. 182 (1940).

Kokate, C.K., *Practical Pharmacognosy*, 1st ed., Vallabh Prakashan, New Delhi, pp. 15-30 (1986a).

Kokate, C.K., *Practical Pharmacognosy*, 1st ed., Vallabh Prakashan, New Delhi, pp. 111 (1986b).

Kokoski, J., Kokoski, R., and Slama, F.J., Fluorescence of powdered vegetable drugs under ultraviolet radiation, *J. Am. Pharmacol. Assoc.* **47**, 715 (1958).

Singh V.K. and Abrar Khan M., Medicinal plants and Folklores: A *Strategy towards conquest of human ailments*, Today and tomorrow's printers and publishers, New Delhi, pp. 4-8 (1990).

WHO/ PHARM/ 92.559/ rev.1., *Quality Control Methods for Medicinal Plant Materials*, Organisation Mondiale De La Sante, Geneva, pp. 9, 22-34 (1992).

(Accepted November 20, 2008)