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A PHARMACOGNOSTICAL REPORT ON *Careya arborea* Roxb. (Lecythidaceae) FRUITS

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ABSTRACT

***Plan**: Careya arborea is a medicinal plant used in Ayurveda and Chinese medicine. The bark, leaves and fruits are used in these systems for the treatment of ulcers, haemorrhoids and tumours. Several reports are available on the pharmacognosy of the leaf and bark of this medicinal tree. However, there is complete lack of information on the pharmacognosy of the fruit. The present paper is the first report on this subject.*

***Methodology:** Histology of the fruit and microscopy of the fruit powder were studied. Tests for identification of phytochemical compound classes were carried out on methanol extract of the fresh fruit. The chemical constitution of the fruits was studied using HPTLC and HPLC.*

***Outcome:** Phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, tannins, sterols and fixed oils. The fruit contains high amount of phenols, of which, gallic acid is present at the rate of 0.92 ± 0.03%. The high content of gallic acid and reported antibacterial activities of the extract quality the fruit for further investigations.*

1. INTRODUCTION

Careya arborea is a medicinal plant used in Ayurveda and Chinese medicine. The bark, leaves and fruits are used in Ayurveda in the treatment of ulcers, haemorrhoids, tumours etc[1]. In Chinese medicine the tree is known as *Ka Li Yu Rui* [2].The tree grows in deciduous forests and grasslands of India. Known as *Katabhi* in Sanskrit, it is a medium sized deciduous tree growing up to a height of 15 meters, with thick, dark grey bark having shallow cracks (Figure 1). Flowers are yellowish white, large and foul-smelling. They occur in thick, swollen, hard, terminal spikes[1].

Figure 1. Careya arborea tree

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The tree has remarkable ability to survive forest fire [3]. Several reports are available on the pharmacognosy of leaf [4,5] and bark [5-7] of this medicinal tree. However, there is complete lack of information on the pharmacognosy of the fruit. The present study was undertaken to fill this lacuna.

2. MATERIAL AND METHODS

Mature fruits from a *Careya arborea* tree growing in the campus of CARe Keralam Ltd (10^0 17' 42" N and 76^0 19' 09 E) were collected. The botanical identity of the tree was established by Dr. K.C. Chacko, on the basis of a herbarium deposited at CARe Keralam Ltd (No. CAReK 101/herb/14). A sample fruit was photographed and used for histological study. Histology of the fruit was studied by taking free hand sections according to Trease and Evans[8]. All the remaining fruits were chopped into small pieces, dried under shade and finally pulverized in a food blender. The powder was used for powder microcopy according to Wallis[9].

2.1. Method of extraction

50 g of freshly chopped fruit were taken in a 500 ml standard flask and soaked in 200 ml of aqueous methanol (1:1). After standing for overnight the solvent was filtered using Whatman filter paper. The filtrate was evaporated and the residue (yield nearly 5%) was used for phytochemical screening. The following tests were carried out:

2.2. Phytochemical screening

34.56 g of freshly chopped fruit were taken in a 500 ml standard flask and soaked in 200 ml of aqueous methanol (1:1). After standing for overnight the solvent was filtered using Whatman filter paper. The filtrate was evaporated and the residue was used for phytochemical screening. The following tests were carried out:

2.2.1. Test for Alkaloids

Mayer's Test: A small quantity of the extract was treated with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with Mayer's reagent. Formation of creamy precipitate indicated the presence of alkaloids. *Dragendorff Test*: A few drops Dragendorff reagent were added to 2-3 ml of the extract. Formation of reddish brown precipitate indicated the presence of alkaloids.

2.2.2. Test for Phenols

Ferric chloride test: 1ml of 5% ferric chloride solution was added to 5 ml solution of extract. Greenish black precipitate indicated the presence of phenols.

2.2.3. Test for flavonoids

A few drops of NP reagent were added to a few ml of extract. Formation of yellow fluorescence that intensified in a few seconds indicated the presence of flavonoids.

2.2.4. Test for Tannins

Ferric chloride test: 1ml of 15% ferric chloride solution was added to 5 ml solution of extract taken in a test tube. Greenish black precipitate indicated the presence of tannins. *Lead acetate test*: 1ml of extract was treated with a few ml of lead acetate solution. Appearance of a white precipitate indicated the presence of tannins.

2.2.5. Test for Phytosterols

Lieberman-Burchard's Test: Mixed 2ml extract with chloroform. Added 1-2 ml acetic anhydride and 2 drops of concentrated H_2SO_4 from the side of the test tube. The mixture first turned red, then blue and finally green indicating the presence of sterols.

2.2.6. Test for fixed oil

Spot test: Pressed small quantity of extract between the folds of a filter paper. Oil stains on the filter paper indicated the presence of fixed oil [10, 11].

2.3. HPTLC analysis of the methanolic extract of Careya arborea fruits

6µl of the methanolic extract were spotted on precoated silica gel HPTLC plate (5 x 10 cm, 0.2 mm thickness, 5-6 mm particle size) as bands of 6 mm width using a Linomat 5 sample applicator fitted with a 100 µl Hamilton syringe. The plate was developed to a distance of 85 mm using a mobile phase consisting of toluene: acetone: formic acid (4.5:4.5:1) , in a CAMAG (Muttenz, Switzerland) twin-trough development chamber, lined with filter paper and presaturated with 30 ml of mobile phase for 20 minutes. The developed plate was dried using a hair drier and scanned. The plate was photographed using CAMAG documentation system Digistore 2 at white light, UV 254 nm and UV 366 nm. A spectrodensitometer (CAMAG) equipped with win CATS planar chromatography manager (Version 1.4.6) software was used for densitometric measurements, spectra recording and data processing. The developed plate was sprayed with 20% sodium carbonate solution followed by Folin-Ciocalteau reagent and dried in a hot air oven. After derivatization the plate was photo documented in day light mode to reconfirm the presence of phenols.

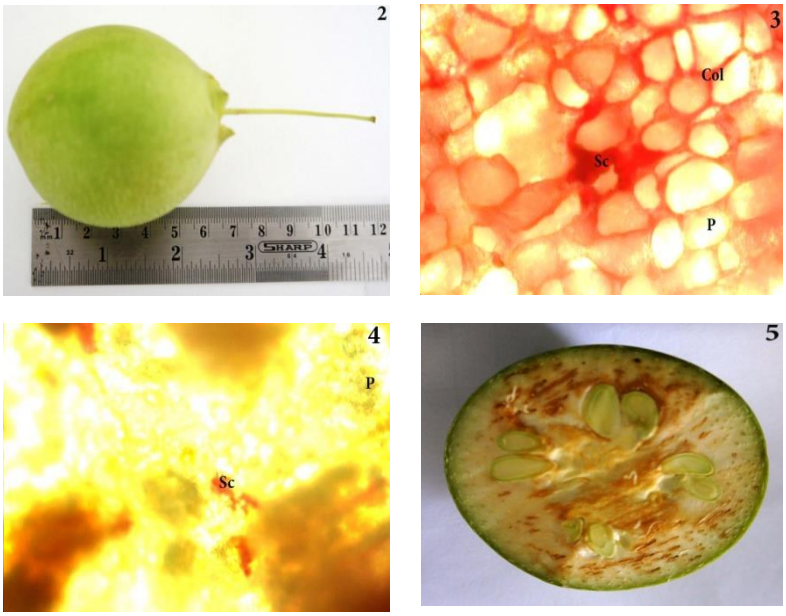
2.4. HPLC analysis of the methanolic extract of Careya arborea fruits

Fruits were chopped into pieces, dried and pulverized. The fruit powder was extracted with pure methanol and the extract recovered. 40 mg of the extract was dissolved in HPLC grade methanol, sonicated and made up to 50 ml. 10 mg of gallic acid standard (Sigma - Aldrich, Lot No. MKBH 8440V) was dissolved in HPLC grade methanol and made up to 50 ml. HPLC analysis was performed on Agilent 1260 Infinity instrument equipped with an auto injector and variable wavelength detector. HPLC data were processed with Chemstation software. Separation was achieved on C18-reversed-phase column (250 mm x 4.6 mm, 5µm) following a variation of the method reported by Sawant et al[12]. The mobile phase consisted of 0.1% orthophosphoric+ acid in water (A) and methanol (B). The gradient was as follows: 95% (A) and 5% (B) for 10 minutes.

The flow rate was 1.0 ml/min and aliquot of 10µl was injected. The UV detection wavelength was set at 272 nm. The method was validated for linearity, accuracy, precision, repeatability, selectivity and specificity. The limit of detection (LOD) was 3.42 µg/ml and limit of quantification (LOQ) was 10.36 µg/ml.

3. RESULTS AND DISCUSSION

Fruits of *Careya arborea* are large, globose, green, glabrous berries, crowned with persistent calyx and style (Figure 2). Persistent calyx is a distinguishing feature of Lecythidaceae. The weight of the fruits ranged from 81.58 g -154.01g.The fruit consists of an upper layer of epidermis. It is parenchymatous with a waxy coating. This is followed by the hypodermis which is of collenchymatous nature. Below is found parenchyma, which is loosely arranged. Vascular bundles are scattered in the parenchyma cells. In between some parenchyma cells are found stone cells, which offer mechanical support (Figure 3).When powder of fruit is macerated with HCl and treated with phloroglucinol, stone cells acquire pinkish red colour (Figure 4).Ovules are arranged in axial placentation with four locules (Figure 5).



Figures 2-5. 2 Fresh fruit of *Careya arborea*, 3 Section of fruit (Col= collenchyma, Sc = stone cell, P = parenchyma), 4. Powder microscopy of fruit (Sc = stone cell, P = parenchyma), 5.Cross section of fruit showing locules.

Phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, tannins, sterols and fixed oils (Table 1).

Table I: Behaviour of methanol extract of *Careya arborea* with different reagents

Reagents	*Color/precipitate*	*Constituents*
Mayer's reagent	No precipitate	Alkaloids absent
Dragendorff reagent	No precipitate	Alkaloids absent
5% Ferric chloride test	Greenish black precipitate	Phenols present
N P reagent	Yellow fluorescence	Flavonoids present
15% Ferric chloride test	Greenish black precipitate	Tannins present
Aq. Lead acetate	White precipitate	Tannins present
Lieberman-Burchard's Test	Reddish brown colour	Sterols present
Spot test	Stains observed	Fixed oils present

The predominant phenol present in *Careya arborea* fruits is gallic acid. On derivatization with Folin-Ciocalteau reagent the gallic acid present in the methanolic extract gets intensified (Figs. 6 and 7).

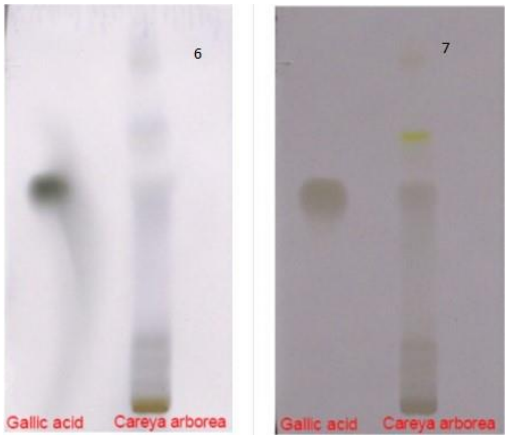


Figure 6. HPTLC plate with gallic acid and methanolic extract before derivatization, Figure 7. After derivatization

Figures 8 and 9 show peak densitogram of methanolic extract of *Careya arborea* in comparison with gallic acid standard. Methanolic extract of the fruit contained high quantities of phenols (1, 2, 3, 4, 6, 7, of Figure 9) along with gallic acid (see peak table, Table 2).

Gallic acid was quantified in the methanolic extract of *Careya arborea* using gallic acid standard. A representative chromatogram is provided in Figure 8. Gallic acid in the sample was quantified and confirmed by injections of three replicates. The methanolic extract of the fruit was found to contain 0.92 ± 0.03% of gallic acid.

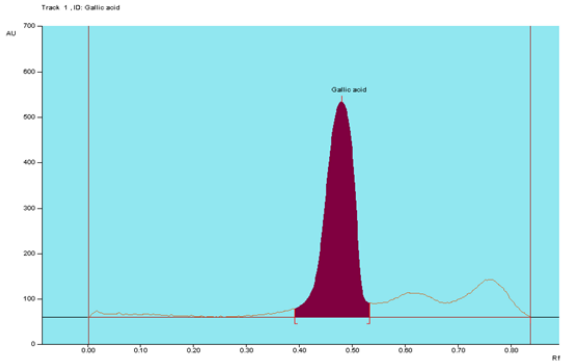


Figure 8: Track 1. Peak densitogram of gallic acid standard scanned at 254 nm before derivatization.

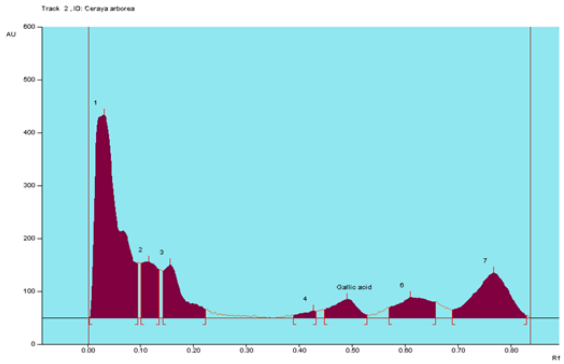


Figure 9: Track 2. Peak densitogram of methanolic extract of *Careya arborea* scanned at 254nm before derivatization

Table 2: Peak table of methanolic extract of *Careya arborea*

Track	*Peak*	*Rf*	*Height*	*Area*	*Assigned substance*
1	1	0.49	474.0	27515.2	Gallic acid standard
2	1	0.03	384.4	18249.4	Unknown
2	2	0.12	106.2	3364.7	Unknown
2	3	0.16	100.6	3964.7	Unknown
2	4	0.43	13.3	384.9	Unknown
2	5	0.49	35.1	1731.3	Gallic acid
2	6	0.61	39.1	2601.0	Unknown
2	7	0.77	85.4	5873.8	Unknown

The ethanol, ethyl acetate and hexane extracts of *Careya arborea* fruits are known to act on *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* [13]. The high content of gallic acid and antibacterial activity qualify this fruit for further investigations.

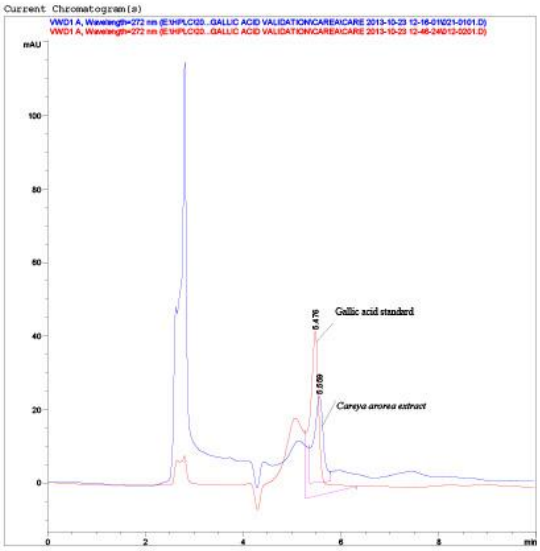


Figure 10: Chromatogram of quantification of gallic acid in methanolic extract of *Careya arborea* using gallic acid standard and VWD detector at 272nm

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