Pharmacognostic standardization and HPTLC fingerprint profile of *Careya arborea* Roxb. stem bark

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Abstract

To ensure reproducible quality of herbal products, proper control of raw material is important. The first step towards ensuring quality of starting material is pharmacognostic standardization. Thus, in recent years, there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance. Careya arborea Roxb. is one of the important medicinal plants belonging to family Lecythidaceae. The present work has been designed to evaluate the pharmacognostic and phytochemical parameters of the stem bark of Careya arborea Roxb for the standardization. Various standardization parameters like macroscopic characters, microscopic evaluation, fluorescence analysis, histochemical study, physicochemical evaluations, preliminary phytochemical screening were carried out according to Ayurvedic Pharmacopeia of India (API) and WHO guidelines and the qualitative parameters were reported. HPTLC fingerprint profile was also evaluated by standard method.

Microscopy of the bark showed the presence of different type of calcium oxalate crystals, stone cells, sclerids and lignified fibres. Total ash value, acid soluble ash, water soluble ash, alcohol soluble extract, water soluble extract, loss on drying and swelling index were found to be 12.03 ± 0.25, 0.8 ± 0.002, 8.43 ± 0.012, 23.88 ± 0.01,21.96 ± 0.02,7.82 ± 1.56,3.33 ± 0.47 respectively. Phytochemical analysis of different extracts showed the presence of aleurone grains, carbohydrates, fats and fixed oils, mucilage, starch, alkaloids, glycosides, saponins, anthraquinones, essential oils, tannins, steroids, triterpenoids and flavonoids.

These studies suggested that the observed pharmacognostic, physicochemical and phytochemical parameters provide referential information for correct identification, authentication of the plant material, formulation development.

Keywords: *Careya arborea* Roxb., Stem bark, Pharmacognostic standardization, Physicochemical, Phytochemical, HPTLC

Introduction

Herbal medicines are comprised of products derived from plant resources and are used for treating and promoting human health. Even the quality of herbal medicines must be controlled just as that of the chemically synthesized ones. Unfortunately, herbal drugs are not regulated as strictly as synthetic drugs. Consequently, the quality standards of herbal products are being decreased by intentional and unintentional adulteration, spurious drugs, substitution of drugs and many other ways that are prone to lowering the quality of herbal products that are marketed and used for healthy survival.

Instead, it causes hazardous effects on consumers' health. Therefore, the quality standards of herbal drugs and products need to be controlled to benefit mankind.[1]

In accordance with the process of standard setting of herbal drugs in the pharmacopeia and other standard texts, the identifications (adulterants and genuine drug), macroscopic (shape and markings), microscopic investigation (qualitative and quantitative), physicochemical parameters (moisture content, acid insoluble ash, water soluble ash, extractive values), phytochemical scheme and other parameters can contribute to quality control of herbal drug and its authentication in the future research.[2-4]

Modern analytical techniques such as Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), Quantitative Thin Layer Chromatography (QTLC) and High-performance Thin Layer Chromatography (HPTLC) have become indispensable in order to accept Ayurveda and traditional herbs around the world.[5] The results from these sophisticated techniques provide a chemical fingerprint to determine the nature of chemicals or impurities present in the plant or extract.[6,7] HPTLC provides improved resolution and also estimation of active phytoconstituents can be carried out with reasonable accuracy in less time.

Moreover, the HPTLC technique has several advantages over other techniques[8,9] like necessitating small amount of sample for analysis, low sensitivity to impurities, wide selections of mobile phases and derivatizing agents. No possibility of interference with previous analysis as fresh stationary phase is used for each analysis. HPTLC is still the most widely used planar chromatographic method and recognized as a tool for fingerprint analysis of complex mixtures of plant extracts.[10,11] An accurate and complete pharmacognostical and phytochemical assessment can

provide scientific basics of the quality of traditional herbs and ayurvedic products. In the present work, an important medicinal plant *Careya arborea* Roxb. is selected which is globally found in India, Ceylon, Malay Penninsula, Cambodia and Australia up to an altitude of 1500 meters.[12,13]

The bark is acrid, astringent, bitter, thermogenic, alexiteric, expectorant, anthelmintic, antipyretic and antipruritic and is useful in tumours, cough, bronchitis, catarrh, skin diseases, urinary discharges, piles, dyspepsia, colic, haemorrhoids, intestinal worms, dysentery, urorrhea, sores, leukoderma, epileptic fits and eruptive fevers particularly smallpox.[12,14]

In snake bite, the fresh bark is applied to the bitten part and an infusion of the same is taken orally. In combination with other drugs the bark is prescribed for snake bite. Dried stem bark is one of the most important components of medicated water "vethuvellam" used by a woman to take a bath after delivery to rejuvenate the body.[15] Fresh bark paste is applied over affected part and infusion of the fruit taken orally for quick relief to scorpion sting.[16]

The main aim of the present investigation was to study the macroscopic, microscopic, histochemical, fluorescence and physicochemical standards of *Careya arborea* Roxb. bark. Phytochemical screening and High-Performance Thin Layer Chromatography (HPTLC) studies have also been done as per WHO guidelines (2011).[17]

Material and Methods

Stem bark was collected from Badlapur, Mumbai (Maharashtra) and authenticated from Agharkar Research Institute, Pune (Maharashtra, India). Bark was air dried and after drying, it was ground into powder and stored in an airtight container at room temperature for further studies.

Macroscopic, microscopic study and physicochemical constants such as the percentage of total ash, acid insoluble ash, water soluble ash; water soluble and alcohol soluble extractive values were calculated according to the methods described in Indian Pharmacopoeia.

Preliminary phytochemical analysis was performed as described by Khandelwal et al[10] and Kokate et al[12]. Fluorescence analysis was conducted using methods of Kokoski et al[13] and Chase and Pratt.[6] Phytochemical analysis was carried out using High Performance Thin Layer Chromatography as per methods described by Wagner and Bladt method.[20]

A qualitative densitometric HPTLC analysis was performed with methanolic extract for the development of characteristic fingerprint profile, which may be used for quality evaluation and standardization of the drug. 10 µl of extract was spotted on pre-coated silica gel 60 F_{254} HPTLC plates (Merck) with the help of CAMAG Linomat V applicator. The plate was developed in a glass twin trough chamber (20 cm × 10 cm) pre-saturated with mobile phase (Toluene: Chloroform: Ethyl alcohol in the ratio 4:4:1). The plate was derivatized using Anisaldehyde sulphuric acid and scanned using TLC Scanner 3 (CAMAG).

Results and Discussion

Pharmacognostic and phytochemical studies are essential for many reasons upholding the botanical identity and lay down standardization parameters which will help and prevent adulterations. Such studies will facilitate authentication of the plants and will confirm reproducible quality of herbal products which will lead to safety and efficacy of natural products.[23] The present work was carried out to report various necessary pharmacognostical standards of *Careya arborea* Roxb. bark.

Macroscopic study: Bark was thick, dark grey in color; inner surface is reddish brown and rough in texture with shallow cracks (Fig. 1).

Organoleptic evaluation: Organoleptic characters of *Careya arborea* Roxb. bark is described in table 1.

Figure 1: Macroscopic characteristics of *Careya arborea* Roxb. bark and bark powder

Table 1
Organoleptic evaluation of *Careya arborea* Roxb. bark powder

S.N.	Parameters	Observation
1	Colour	Pinkish brown
2	Texture	Smooth
3	Taste	Astringent
4	Odour	Not characteristic

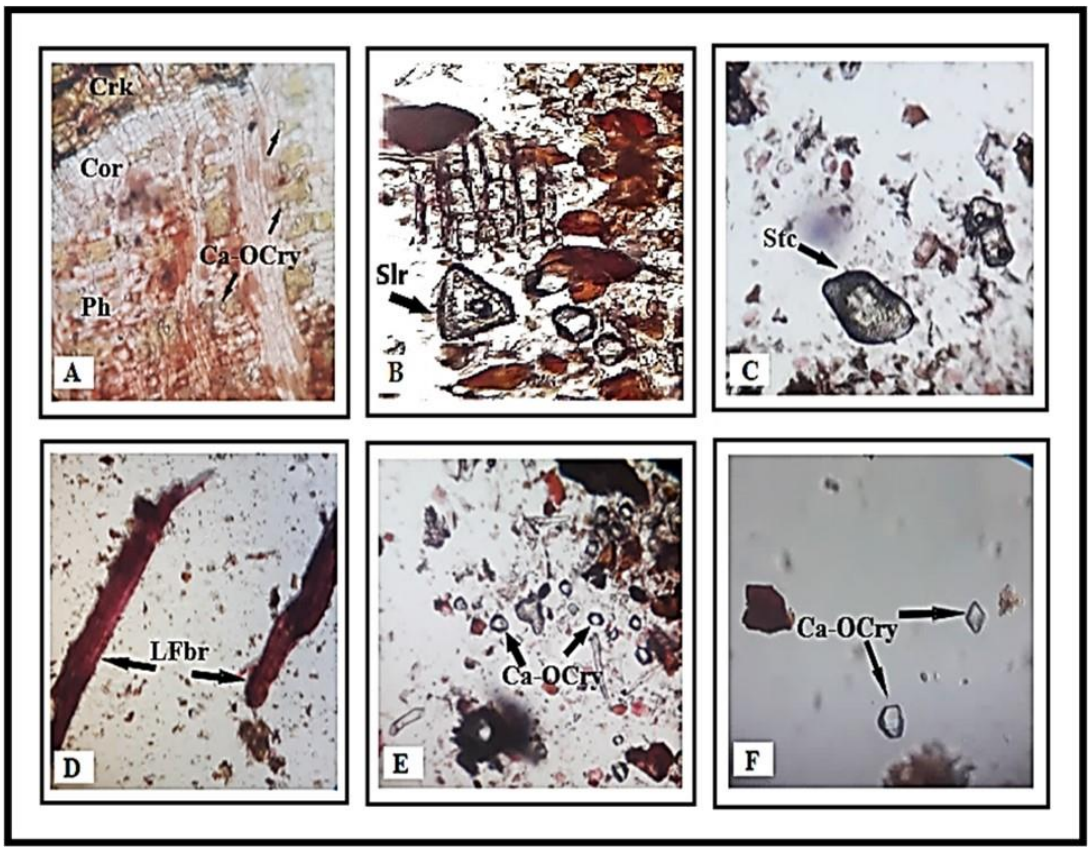

Figure 2: Microscopic characteristics of *Careya arborea* Roxb. bark
Keywords: A- T.S. of bark under compound microscope (10X) (Crk: Cork; Cor: Cortex; Ph: Phloem; Ca-OCry: Calcium oxalate crystals), Powder microscopy showing B-Sclerids (Slr), C- Stone cells (Stc), D- Lignified fibers (LFbr), E and F- Calcium oxalate crystals (Ca-OCry)

Microscopic study: For microscopic study, transverse sections of bark were taken and observed under 10X of compound microscope. Transverse section of bark showed distinct cork region, cortex and secondary phloem region. The cork region was multi-layered (10 - 16), consisting of thick walled, rectangular and blackish brown colored cork cells. Cork was followed by multilayered cortex. Cortex cells were parenchymatous with rectangular to polygonal in shape. Some cells were found to be golden yellow in color with yellowish contents. Secondary phloem was made up of phloem parenchyma, 1-2 uniseriate or biseriate medullary rays and fibers.

The fibers were round in shape and formed round patches. Rhomboidal and prismatic calcium oxalate crystals were present in cortex cells and phloem parenchyma. The powdered bark was studied for anatomical markers. Powder microscopic analysis of bark revealed the presence of calcium oxalate crystals, stone cells, sclerids and lignified fibres (Fig. 2).

Histochemical study: In the present histochemical study, tannins, lignin, calcium oxalate crystals, aleurone grains, stone cells, alkaloids and steroids were detected in the bark.

Fluorescence analysis: Fluorescence analysis of *Careya arborea* Roxb. bark powder was carried out; change in the color was noted down and tabulated in table 3. The fluorescence analysis of various extracts of *Careya arborea* Roxb. bark was also tabulated in table 4.

Physicochemical analysis: Physicochemical constants such as the percentage of foreign matter, loss on drying, swelling index, total ash, acid insoluble ash, water soluble ash, water soluble and alcohol soluble extractive values were estimated for *Careya arborea* Roxb. bark (Table 5).

Phytochemical evaluation: In the present investigation, preliminary phytochemical analysis of *Careya arborea* Roxb. bark was performed using various solvents (Petroleum ether, Chloroform, Methanol and Water) for extraction and the results are depicted in table 6. In the present study, *C. arborea* bark extracts showed the presence of primary metabolites viz. aleurone grains, carbohydrates, fats and fixed oils, mucilage and starch and secondary metabolites viz. alkaloids, glycosides, saponins, anthraquinones, essential oils, tannins, steroids, triterpenoids and flavonoids (Table 6).

Table 2
Histochemical analysis of *Careya arborea* Roxb. bark

S.N.	Reagents	Test	Observation	Inference
1	Iodine	Starch	Blue colour	-
2	Ferric Chloride	Tannin	Bluish black colour	+
3	Dil HCl+ Phloroglucinol	Lignin	Pink colour	+
4	Conc. HCl	Calcium Oxalate crystals	Insoluble in acetic acid; Dissolve in HCl	+
5	Ruthenium Red	Mucilage	Pink colour	-
6	Sudan Red III	Oil globules	Red colour	-
7	Alco. Picric acid	Aleurone grains	Yellowish brown colour	+
8	Conc. H_2SO_4	Stone cells	Green colour	+
9	Dragendorff's Reagent	Alkaloids	Light orange colour	+
10	Libermann Burchard Reagent	Steroids	Green colour	+

Keywords: '+' - Detected; '-' - Not detected

Table 3
Fluorescence analysis of *Careya arborea* Roxb. bark powder

S.N.	Fluorescence tests	Observation under		
		Visible light	UV 254 nm	UV 366 nm
1	Powder as such	Light Brown	Black	Black
2	Powder + 1N NaOH in methanol	Black	Black	Black
3	Powder + 1N HCl	Brown	Dark Brown	Black
4	Powder + 1N NaOH in water	Dark Brown	Black	Black
5	Powder + HNO_3 (1:1)	Light Brown	Dark Brown	Dark Brown
6	Powder + H_2SO_4 (1:1)	Brown	Dark Brown	Dark Brown
7	Powder + 1% Picric acid	Yellow	Fluorescent Green	Fluorescent Green
8	Powder + 5% Iodine	Brown	Dark Brown	Dark Brown
9	Powder + 5% $FeCl_3$	Black	Black	Black
10	Powder + 25% NH_3 + HNO_3	Yellow	Dark Brown	Black
11	Powder + Conc. HNO_3	Yellow	Dark Green	Dark Brown
12	Powder + 10% $K_2Cr_2O_7$	Black	Black	Black
13	Powder + 50% KOH	Brown	Black	Black
14	Powder + Methanol	Dark Brown	Dark Brown	Black
15	Powder + Ethanol	Brown	Dark Brown	Black
16	Powder + Toluene	Brown	Dark Brown	Black
17	Powder + Glacial acetic acid	Dark Brown	Black	Black

Table 4
Fluorescence analysis of *Careya arborea* Roxb. bark extracts

S.N.	Extracts	Observation under		
		Visible light	UV 254 nm	UV 366 nm
1	Aqueous	Brown	Brown	Dark Brown
2	Methanol	Reddish Brown	Greenish Brown	Dark Brown
3	Ethanol	Reddish Brown	Dark Brown	Yellowish Brown
4	Petroleum Ether	No Fluorescence	No Fluorescence	No Fluorescence
5	Benzene	No Fluorescence	No Fluorescence	No Fluorescence
6	Chloroform	No Fluorescence	No Fluorescence	No Fluorescence
7	Acetone	Brown	Greenish Brown	Greenish Brown

HPTLC Fingerprint profile: In the present study, characteristic HPTLC fingerprint profile was developed for *Careya arborea* Roxb. bark. The developed HPTLC fingerprint profile (Fig 3) showed that the methanolic bark extract of *C. arborea* contains a variety of phytocompounds and their respective Rf values were presented in table 7.

Table 5
Physicochemical parameters for *Careya arborea* Roxb. bark

S.N.	Physicochemical Parameters	Result (Mean ± SD)
1	Foreign Matter (% w/w)	3.13 ± 1.09
2	Loss on drying (% w/w)	7.82 ± 1.56
3	Swelling Index (ml)	3.33 ± 0.47
5	Ash values	
	a. Total ash value (% w/w)	12.03 ± 0.25
	b. Acid insoluble ash value (% w/w)	0.8 ± 0.002
	c. Water soluble ash value (% w/w)	8.43 ± 0.012
6	Extractive values	
	a. Alcohol Soluble (% w/w)	23.88 ± 0.01
	b. Water Soluble (% w/w)	21.96 ± 0.02

Table 6
Preliminary phytochemical analysis of *C. arborea* Roxb. bark extracts

S.N.	Phytoconstituents	PE	CE	ME	AE
1	Acid compounds	ND	ND	ND	ND
2	Aleurone Grains	+	+	+	+
3	Amino acids	ND	ND	ND	ND
4	Carbohydrates	ND	+	+	+
5	Fats and Fixed oils	+	+	ND	ND
6	Proteins	ND	ND	ND	ND
7	Starch	ND	+	+	+
8	Alkaloids	ND	+	ND	+
9	Anthraquinones	+	+	ND	ND
10	Essential Oils	ND	ND	ND	+
11	Flavonoids	ND	ND	+	ND
12	Glycosides	ND	ND	ND	+
13	Mucilage	ND	ND	+	+
14	Resins	ND	ND	ND	ND
15	Saponins	ND	ND	ND	+
16	Steroids	+	+	+	ND
17	Tannins	ND	ND	+	+
18	Triterpenoids	ND	ND	+	+

Keywords: PE – Petroleum ether extract; CE- Chloroform extract; ME- Methanolic extract;
AE- Aqueous extract; + - Detected; ND - Not Detected

Table 7
HPTLC fingerprint profile (Rf values) of Methanolic extract of *Careya arborea* Roxb. bark

S.N.	Before Derivatization	After Derivatization	
	254 nm	366 nm	540 nm
1	0.05	0.27	0.005
2	0.61	0.51	0.04
3	0.86	0.56	0.15
4	-	0.59	0.29
5	-	0.63	0.52
6	-	0.69	0.59
7	-	0.89	0.64

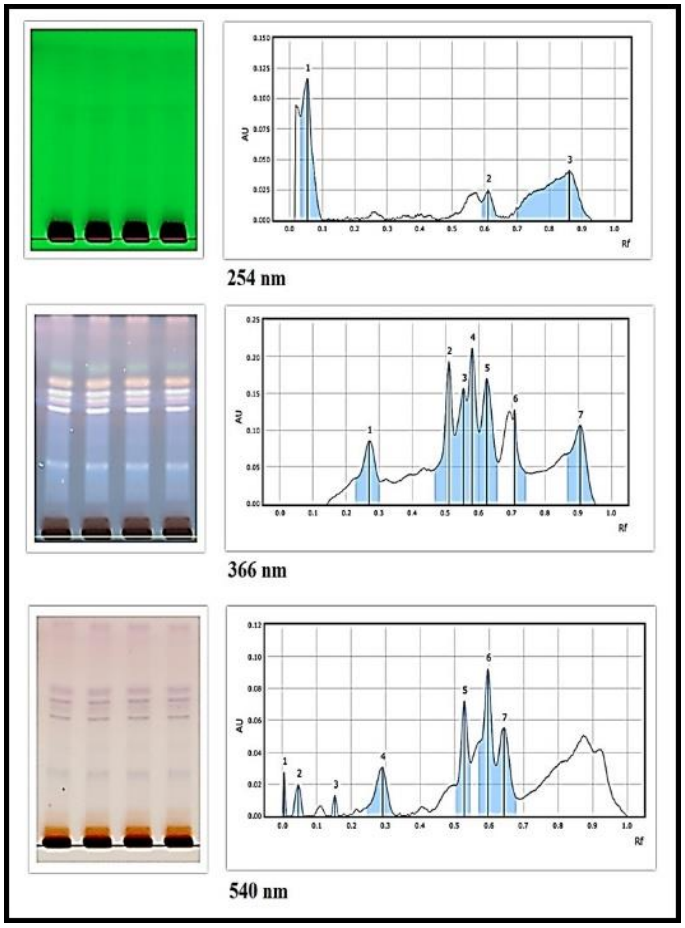

Figure 3: High Performance Thin Layer Chromatography fingerprint profile of *Careya arborea* Roxb. bark extract

Conclusion

The present study endeavors a modest comprehensive examination of the *Careya arborea* Roxb. bark. Since the entire plant of *Careya arborea* Roxb. has therapeutic qualities, the present investigation has laid down a set of anatomical features, fluorescence characters and physicochemical parameters for the bark which can be employed for its botanical identification and standardization of *Careya arborea* Roxb. This study could be useful in the preparation of the monograph of *C. arborea* bark in the pharmacopoeia.

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