Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(1): 1730-1735
Received: 07-11-2018
Accepted: 09-12-2018

Priyanka Gupta
Dept. of Biotechnology, B. K. Birla College of Arts, Science & Commerce (Autonomous), Kalyan (West), Maharashtra, India

Darshana Patil
Dept. of Botany, Smt. C.H.M. College, Ulhasnagar, Maharashtra, India

Avinash Patil
Dept. of Biotechnology, B. K. Birla College of Arts, Science & Commerce (Autonomous), Kalyan (West), Maharashtra, India

Correspondence
Priyanka Gupta
Dept. of Biotechnology, B. K. Birla College of Arts, Science & Commerce (Autonomous), Kalyan (West), Maharashtra, India

Quality evaluation and high performance thin layer chromatography fingerprint profile of *Careya arborea* Roxb. seeds

Priyanka Gupta, Darshana Patil and Avinash Patil

Abstract
Objective: *Careya arborea* roxb. Seeds are useful in traditional medicine for the treatment of various ailments such as ulcers, wound healing, dermatopathies, colic and loose motions and hence it is important to standardize it for use as an herbal drug. The current study provides requisite pharmacognostic details about *C. arborea* seeds.
Methods: *C. arborea* seeds are subjected to organoleptic, macroscopic and microscopic examination, physicochemical constants determination, fluorescence study, preliminary phytochemical analysis for quality evaluation. HPTLC fingerprint profile is also developed for *Careya arborea* seeds.
Results: Powder microscopy of *Careya arborea* seeds showed stone cells, spiral thickening of vessels, fibres and presence of abundant starch grains. The water soluble extractive value was found to be higher as compared to alcohol soluble extractive value. Preliminary phytochemical screening revealed the presence of aleurone grains, carbohydrates, saponins, steroids, mucilage and starch. A developed HPTLC fingerprint profile of methanolic seed extract is unique for the seed of *C. arborea* and can be used as identifying marker.
Conclusion: Pharmacognostic details and HPTLC fingerprint profile of the seeds of *C. arborea* will provide referential information for correct identification, standardization and updating of monograph and will be helpful to ensure the purity, safety and efficacy of the seeds.

Keywords: *Careya arborea*, Seeds, pharmacognosy, physicochemical constants, fluorescence analysis, preliminary phytochemical analysis, HPTLC fingerprint profile

Introduction
The popularity of plant based drugs is increasing all over the world particularly in the developed countries because of their wide therapeutic effects and less side effects than synthetic drugs but one of the obstacles in its acceptability is the lack of standardization. Therefore, there is a need to develop standardization parameters of the plant materials, which are to be used in preparation of various herbal drugs [1]. The exact identification and the quality assurance of the starting raw material are essential to ensure the reproducible quality of herbal drugs, which will ultimately lead to their safety and efficacy [2]. The process of standardization can be achieved by a set of pharmacognostic methods, which will ensure plant identity, authenticity and quality [3]. These methods include macroscopic and microscopic studies, determination of ash values, foreign matter, moisture content, extractive values, swelling index, foaming index, crude fibre, fluorescence studies, qualitative and quantitative chemical evaluation and chromatographic examinations [4, 5].
Careya arborea Roxb. (Family: Lecythidaceae) is one of the important medicinal tree as its most of the parts are used in traditional medicinal purpose. It is named as Kumbhi in Hindi and Slow match tree in English [6]. The tree grows in deciduous forests and in grasslands all over of India. It is also found in Sri Lanka, Malay and peninsula [7]. It is a medium sized tree attains a height of 9 to 18m [8]. Many pharmacological activities of this plant are reported such as antidiarrheal, anti-inflammatory, analgesic, hypoglycaemic, antibacterial, antifungal, antileishmanial, α-glucosidase inhibitory, antioxidant, hepatoprotective, cytotoxic, CNS depressant and anticoagulant [9]. Traditionally, seeds are utilized in treatment of ulcers, dermatopathies, wound healing, colic and loose motions. Seeds are also used as one of the ingredients in "Marma Gutika", an ayurvedic formulation [8].
Some pharmacognostic parameters of *Careya arborea* Roxb. Seeds are already included in Ayurvedic Pharmacopoeia of India. However, the present study is carried out to study additional parameters and also to re-evaluate existing parameters for comparative study. It will make existing pharmacognostic data enrich for authentication and quality control of this drug. Pharmacognostic parameters are not enough in establishing the standards of herbal drugs so

now a days instrumental analysis, which gives a more concrete picture regarding the qualitative and quantitative aspects of bioactive molecules, is widely accepted in the quality evaluation of herbal drugs [10]. In this regard, HPTLC fingerprint has been developed for the first time for *Careya arborea* seeds. Thus, in the present work standardization of *Careya arborea* seeds has been carried out in terms of macroscopy and microscopy characterization, physicochemical evaluation, fluorescence studies, preliminary phytochemical analysis and HPTLC fingerprint profile.

2. Materials and Methods

2.1. Plant material: Fully ripe mature fruits were collected in the month of July from the forest area of Badlapur, Mumbai (India). Seeds were scooped out from them and washed under running tap water. Seeds were authenticated from Agharkar Research Institute, Pune. Fresh mature seeds were used for macroscopic and microscopic studies and remaining seeds were air dried, ground into powder and stored in airtight container for further studies.

2.2. Reagents and Chemicals: All reagents and chemicals used for this study were analytical grade obtained from SDFCL, Mumbai (India) and Himedia, Mumbai (India).

2.3. Macroscopic, microscopic and organoleptic studies- Macroscopic and microscopic characterization of seed and seed powder were carried out according to standard methods [11]. Seeds were macroscopically examined for shape, colour and size as well seed powder was evaluated for organoleptic characters like colour, odour, taste and texture. Free hand sections were taken and then stained with diluted safranin. Sections and powder were examined under compound microscope. It was evaluated and photographed.

2.4. Physicochemical evaluation- Physicochemical parameters such as loss on drying, percentage of total ash, acid insoluble ash, water soluble ash; water soluble and alcohol soluble extractive values, swelling index, foaming index were determined according to the standard methods [12, 13] and the WHO guidelines on quality control methods for medicinal plants [14]. The determinations were performed in triplicates and results were expressed as mean ± SD.

2.5. Fluorescence studies- Fluorescence analysis of powder and seed extracts in different solvents were carried out and observed in visible, UV short (254) and long (366) wavelength regions. Change in the colour of the powder and extracts was noted down [15, 16].

2.6. Preliminary phytochemical screening- Powdered seed was extracted with three solvents of different polarity *viz.* petroleum ether, methanol and water. The extracts were filtered and subjected to qualitative tests for the identification of various phytochemical constituents as per the method reported [17].

2.7. HPTLC fingerprint profile- A qualitative densitometric HPTLC analysis was performed with methanolic seed extract using WINCATS software for the development of characteristic fingerprint profile, which may be used for quality evaluation and standardization of the drug. 10 µl of extract was loaded on precoated TLC silica gel 60 F_{254} plates (Merck) with the help of CAMAG Linomat 5 applicator. The plate was developed to a distance of 70mm using CAMAG twin trough development chamber (10 cm × 20 cm) which was presaturated for 20 minutes with mobile phase (Toluene: Chloroform: Ethyl alcohol in the ratio 4:4:1). The plate was photo documented and scanned by CAMAG TLC scanner 3 at white light, UV 254 nm and UV 366 nm. The plate was derivatized using Anisaldehyde sulphuric acid and visualized using CAMAG TLC visualizer. The plate was again photo documented and scanned.

The developed chromatogram for *Careya arborea* Roxb. seed will be specific with selected solvent system and R*f* values, and serve as better tool for standardization of the drug. Phytochemicals, some of which are having therapeutic value are species specific and can be visualized by developing chromatograms.

3. Results

3.1. Macroscopic characteristics

Fruits are green, large, globose and glabrous berries. It is 8-9 cm in diameter; crowned with persistent calyx and style, which is a characteristic feature of Lecythidaceae family. Five to twenty dark brown seeds are embedded in fleshy pulp of fruit. Seeds are internally creamiest white. They are 2-2.2 cm long and 1-1.2 cm in width. Mature seeds are exalbuminous whereas immature are albuminous. Seeds are oval ellipsoid in shape and indehiscent. Testa is hard which become wrinkled after drying.

3.2. Microscopic characteristics

Transverse section of seed shows an outermost layer of testa, which is composed of sclerenchymatous cells. It is followed by collapsed cells of outer integument with dark brown content. Underneath this inner integument with dark brown content is present. Many layers of thin walled, polygonal parenchymatous cells are present in cotyledon region, which are embedded with a large number of starch grains. Powder microscopy of seed powder showed presence of vessels with spiral thickening, stone cells, fibres and abundant starch grains.

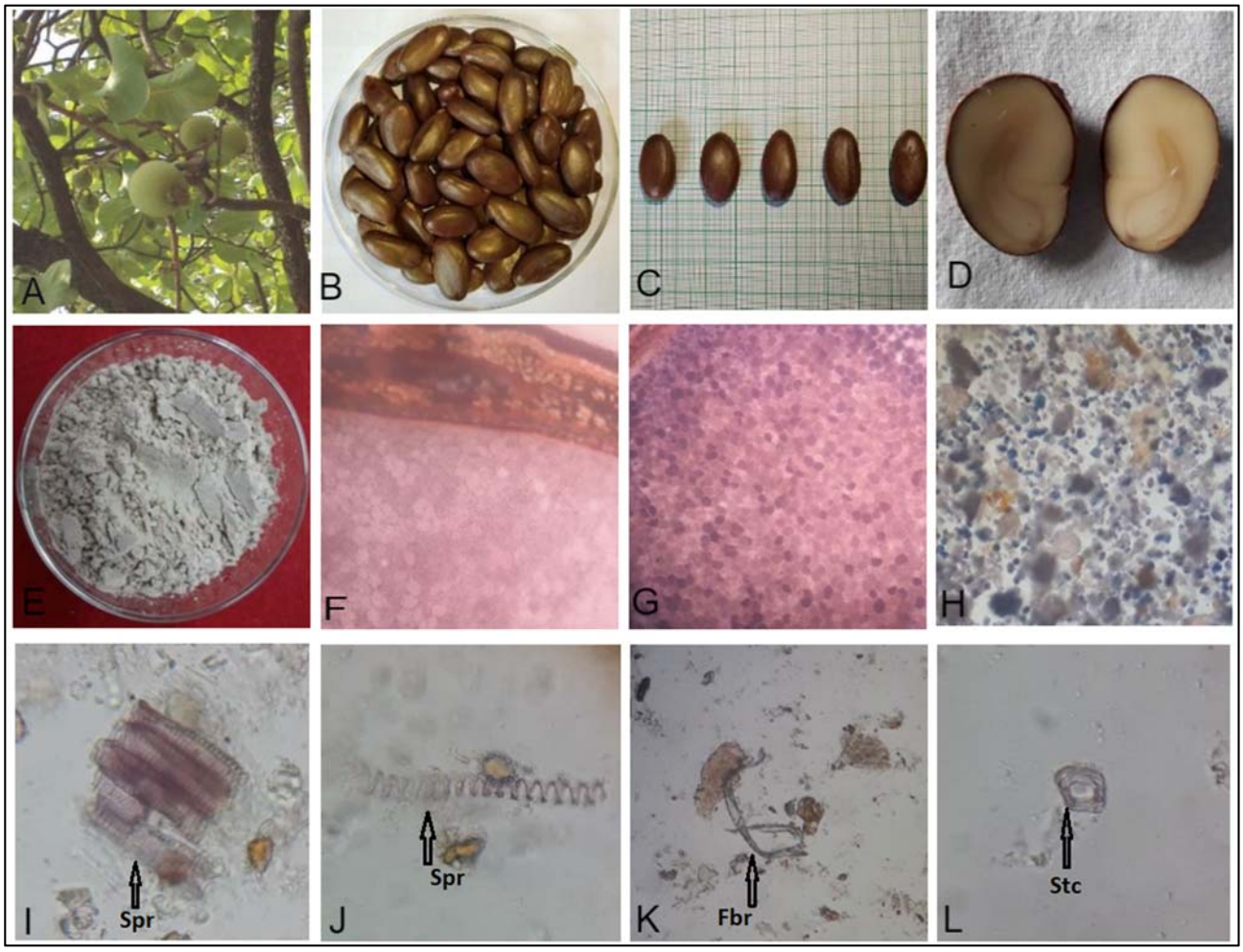

Fig. 1: Macroscopic, microscopic and powder characteristic of *Careya arborea* Roxb. Seeds. A: Fruits on tree, B: Seeds, C: Macroscopy of seeds, D: L.S. of seed showing embryo, E: Seed Powder, F: T.S. of seed under compound microscope(10X), G: T.S. of seed showing starch grains, Powder microscopy showing H: starch grains (10X), I: spiral thickening of vessels (SPR), J: spiral thickening (SPR), K: fibres (FBR), L: stone cell (STC).

3.3. Organoleptic characteristics of powder

Organoleptic evaluation can be done by visual inspection. This evaluation provides the simplest and quickest means to establish the identity and purity and thereby ensure quality of a particular drug [11, 18].

Organoleptic characters of powder are depicted in Table 1.

Table 1: Organoleptic evaluation of *Careya arborea* Roxb. seed powder

Features	Observation
Colour	Creamiest white
Texture	Smooth fine
Taste	Astringent
Odour	Characteristic pleasant

3.4. Physicochemical Evaluation

The determination of physicochemical parameters for a crude drug is helpful in setting standard data for quality control of a crude herbal drug, as these parameters are mostly constant for a medicinal plant. Therefore, these are important for the detection of drug adulteration or improper handling of raw materials [4]. One of such a parameter is ash value which will help in the detection of foreign inorganic matter such as metallic salts and/or silica [19]. The values obtained for ash content (total ash, acid insoluble ash, water soluble ash) of seeds are shown in table 2.

Extractive values are also useful for evaluation of herbal drug. It gives the idea about nature of constituents, extracted with the solvent used for extraction of crude drug [17]. Extractive values for seeds are tabulated in table 2.

Loss on drying is the loss of mass expressed as percent w/w. Loss on drying, swelling index and foaming index for *Careya arborea* Roxb. seeds are in table 2.

Table 2: Physicochemical parameters for *Careya arborea* Roxb. seeds

No.	Physicochemical Parameters	Result (Mean ± SD)
1.	Loss on drying (% w/w)	33.93 ± 0.003
2.	Swelling Index (ml)	1 ± 0.1
3.	Foaming Index	333.33 ± 96.235
4.	Ash values	
	a. Total ash value (% w/w)	2.0 ± 0.006
	b. Acid insoluble ash value (% w/w)	0.7 ± 0.007
	c. Water soluble ash value (% w/w)	1.9 ± 0.003
5.	Extractive values	
	a. Alcohol Soluble (% w/w)	8.8 ± 0.025
	b. Water Soluble (% w/w)	15.68 ± 0.041

3.5. Fluorescence study

Fluorescence analysis of *Careya arborea* Roxb. seed powdered drug and seed extract in different solvents *viz.* water, methanol, ethanol and petroleum ether showed their characteristic fluorescent colour. Change in the colour was examined under long UV (366nm), UV (254nm) and visible light. The results are presented in table 3 and 4.

Table 3: Fluorescence analysis of *Careya arborea* Roxb. seed powder

No.	Treatment	Observation under		
		Visible light	UV 254 nm	UV 366 nm
1.	Powder as such	Creamiest White	Dark Brown	Creamiest White
2.	Powder + 1N NaOH in methanol	Creamiest White	Dark Brown	Fluorescent White
3.	Powder + 1N HCl	White	Dark Brown	Dark Brown
4.	Powder + 1N NaOH in water	White	White	White
5.	Powder + HNO_3 (1:1)	Yellow	Light Green	Light Green
6.	Powder + H_2SO_4 (1:1)	Creamiest White	Dark Brown	White
7.	Powder + 1% Picric acid	Yellow	Dark Green	Dark Green
8.	Powder + 5% Iodine	Black	Black	Black
9.	Powder + 5% $FeCl_3$	Yellow	Dark Green	Dark Green
10.	Powder + 25% NH_3 + HNO_3	Creamiest White	Brown	White
11.	Powder + Conc. HNO_3	Yellow	Dark Green	Dark Green
12.	Powder + 10% $K_2Cr_2O_7$	Yellow	Yellow	Light Green
13.	Powder + 50% KOH	Brown	Dark Brown	Dark Green
14.	Powder + Methanol	Creamiest White	White	White
15.	Powder + Ethanol	White	Dark Brown	Fluorescent White
16.	Powder + Toluene	Brown	Dark Brown	Fluorescent White
17.	Powder + Glacial acetic acid	Creamiest White	Light Brown	Fluorescent White

Table 4: Fluorescence analysis of *Careya arborea* Roxb seed extracts.

No.	Extract	Observation under		
		Visible light	UV 254 nm	UV 366 nm
1.	Aqueous	No fluorescent	No fluorescent	Fluorescent white
2.	Methanol	Pale yellow	Brown	Fluorescent white
3.	Ethanol	No fluorescent	No fluorescent	No fluorescent
4.	Petroleum Ether	No fluorescent	No fluorescent	No fluorescent

3.6. Qualitative preliminary phytochemical analysis

This is also an important parameter, which gives an idea about the presence of various pharmacological bioactive compounds. Analysis of seeds in three different solvents (petroleum ether, methanol and water) showed the presence of carbohydrates, mucilage, aleurone grains, starch, steroids and saponins. The results are depicted in table 5.

Table 5: Preliminary phytochemical screening of *Careya arborea* Roxb. seed extracts

No.	Phytoconstituents	Petroleum ether	Methanol	Water
1.	Acid compounds	ND	ND	ND
2.	Alkaloids	ND	ND	ND
3.	Amino acids	ND	ND	ND
4.	Proteins	+	+	+
5.	Carbohydrates	ND	+	+
6.	Starch	+	+	+
7.	Glycosides	ND	ND	ND
8.	Mucilage	ND	+	+
9.	Tannins	ND	ND	ND
10.	Steroids	+	+	+
11.	Flavonoids	ND	ND	ND
12.	Saponins	ND	+	+
13.	Aleurone Grains	+	+	+
14.	Fats and Fixed oils	ND	ND	ND
15.	Anthraquinones	ND	ND	ND
16.	Resins	ND	ND	ND
17.	Essential Oil	ND	ND	ND

Keywords: ND-Not detected; + Present

3.7. HPTLC Fingerprint profile

The evaluation and quality control of herbal medicines are moving a step ahead towards an integrative and comprehensive direction, in order to tackle the complex herbal drugs. High-performance thin layer chromatography (HPTLC) is one of the sophisticated and advanced instrumental techniques to obtain fingerprint patterns of the herbs and herbal drugs, quantification of active ingredients and detection of adulteration [20].

The WHO also accepts HPTLC fingerprint profile as an identification and quality evaluation technique for medicinal plants since 1991 [21]. HPTLC fingerprint profile of methanolic seed extract showed characteristic band pattern before and after derivatization with anisaldehyde sulphuric acid. R*f* values under different wavelengths before and after derivatization are tabulated in table 6.

Table 6: HPTLC Fingerprint Profile (R*f* values) of methanolic extract of *Careya arborea* Roxb. seed

No.	Before Derivatization	After Derivatization	
	254nm	366 nm	540 nm
1.	0.065	0.28	0.26
2.	0.19	0.47	0.45
3.	0.49	0.52	0.49
4.	0.86	0.55	0.52
5.	-	0.59	0.56
6.	-	0.64	0.88
7.	-	0.73	-
8.	-	0.90	-

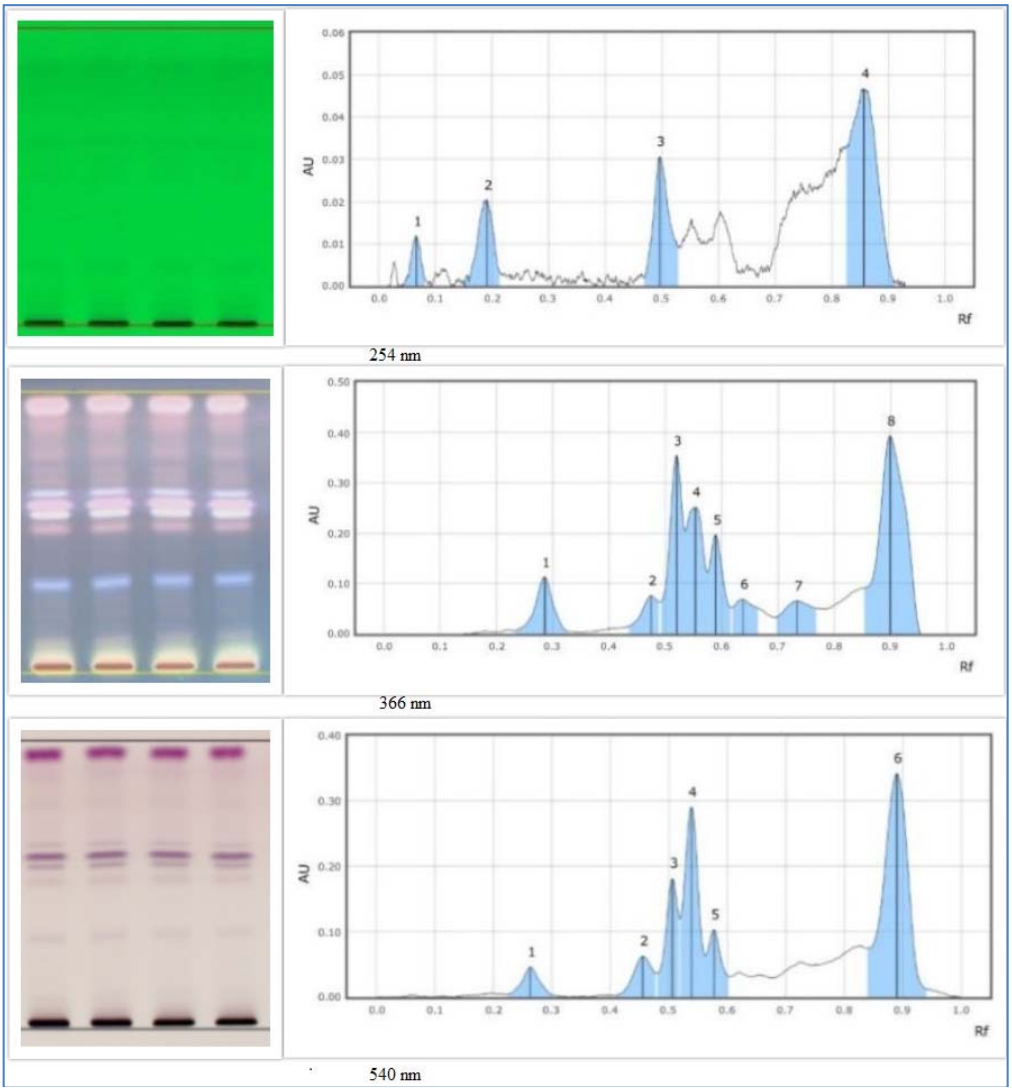

Fig 2: High performance thin layer chromatography finger print profile of *Careya arborea* Roxb. seed extract

4. Discussion

Correct identification and authentication of starting raw material of herbal drugs is necessary before its use as a drug individually or as an ingredient of formulation because the therapeutic efficacy of herbal drugs depends greatly on the use of unadulterated and authenticated raw materials. In this framework, this study could be useful to establish pharmacognostic parameters for seeds of *Careya arborea* Roxb. which may definitely useful in deciding the purity, quality, safety, efficacy and authenticity of this herbal drug in fresh and powdered form. According to WHO the macroscopic and microscopic studies of a medicinal plant is the preliminary step toward establishing its identity and purity and should be carried out before any tests are undertaken [18]. Therefore, macroscopic, microscopic and organoleptic examination of seeds has been done. Macroscopic and organoleptic studies of seed might be useful for distinguishing it from its substitutes and adulterants. Microscopic evaluation allows more detailed examination of the seed and enable to identify the organized structural features.

Physicochemical parameters of the seeds will also serve as standard data for the quality control of the herbal preparation containing these seeds. Using these standards, the plant can be differentiated from other related species [22]. Ash values, water soluble and alcohol soluble extractive values of the seeds are found to be within the API limits. The results for extractive values suggested that the powdered seeds have high water soluble phytochemicals such as carbohydrates and mucilage as compared to alcohol soluble phytochemicals.

Moisture content of crude drug is directly related to its

stability. High moisture increases the chances of microbial contamination and decreases shelf life of a crude drug. Hence, moisture content of a drug should be determined and should be controlled [23]. *Careya arborea* Roxb. seeds have considerable amount of moisture so the seed powder should be stored in proper dried form.

Most of the herbal drugs are of pharmaceutical values due to their swelling properties. Swelling index of seed was possibly due to presence of mucilage and foaming index for seeds shows the presence of high saponin phytoconstituents.

Fluorescence is the phenomenon exhibited due to presence different functional groups in the phytochemical constituents. Chemical constituents may produce fluorescence either in the visible light or in ultra violet light. If the substance themselves do not show fluorescence, they may often be converted into their fluorescent derivatives or decomposition products by treating with different reagents. The fluorescence character of powdered drugs plays a vital role in the determination of adulterants in the drug material [24]. Hence, the reults obtained from the present fluorescent studies will also help to check the impurities present in seed powder and seed extracts of *Careya arborea* Roxb.

Presence or absence of certain phytoconstituents in an plant extract is determined by colour reactions of the compounds with specific chemicals. This procedure is a simple preliminary prerequisite before going for detailed phytochemical investigation [25]. In the present study, preliminary phytochemical analysis reveals the presence of various secondary plant metabolites which have been claimed to be responsible for various pharmacological activities and also reveals that it is an important medicinal plant part to explore for further phytochemical and biological assays.

The unique band pattern obtained from HPTLC fingerprint profile of seed extract may also serve as useful data for identification, quality control and detection of any type of adulteration, especially of similar species of this drug.

Pharmacognostic studies on *Careya arborea* Roxb. seeds is a substantial step and further studies are needed to identify, isolate, characterize and elucidate the structure of bioactive compounds and evaluate pharmacological activity and toxicity of seeds to establish it as a safe and effective drug.

Acknowledgement

The author and co-authors are thankful to Management of B. K. Birla College of Arts, Science and Commerce (Autonomous), Kalyan, Maharashtra (India) for providing necessary research facilities to complete this study.

References

1. Alam F, Najumus Saqib Q. Pharmacognostic standardization and preliminary phytochemical studies of *Gaultheria trichophylla*. Pharmaceutical biology. 2015; 53(12):1711-1718.
2. Nayak BS, Patel KN. Pharmacognostic studies of the *Jatropha curcas* leaves. International journal of pharmtech research. 2010; 2(1):140–143.
3. Ozarkar K.R. Studies on anti-inflammatory effects of two herbs *Cissus quadrangularis* Linn. and *Valeriana wallichii* DC using mouse model, Ph.D. Thesis, University of Mumbai, Mumbai, 2005.
4. WHO. Quality Assurance of Pharmaceuticals: A Compendium of Guidelines and Related Materials, Good Manufacturing Practices and Inspection. Geneva: WHO, 1996a, 2.
5. WHO. Guidelines for the Assessment of Herbal Medicines. WHO Technical Report Series. Geneva: WHO, 1996b, 863.
6. Kirtikar KR, Basu BD. Indian Medicinal Plants, 2nd ed. Lalit Mohan Basu Publications, 1980, 894-895.
7. Gupta PC, Sharma N, Rao CV. Pharmacognostic studies of the leaves and stem of *Careya arborea* Roxb. Asian Pacific journal of tropical biomedicine. 2012; 2(5):404-408.
8. Government of India. The Ayurvedic Pharmacopoeia of India. 5(1):110.
9. Khaliq HA. Pharmacognostic, physiochemical, phytochemical and pharmacological studies on *Careya arborea* Roxb: A review. The J Phyto pharmacol. 2016; 5(1):27-34.
10. Rasheed NMA, Shareef MA, Mushtaq A, Gupta VC, Shamsul A and Shamshad AK. HPTLC finger print profile of dried fruit of *Physalis alkekengi* Linn. Phcog J. 2010; 2(12):464-469.
11. Mukherjee PK. Quality control of Herbal Drugs: An approach to evaluation of botanicals, India: Business Horizons, 2008.
12. Government of India. Indian pharmacopeia. 4th ed. New Delhi: Ministry of Health and Welfare, Controller of Publications, 1996, A53-A54.
13. Government of India. The Ayurvedic pharmacopoeia of India. 1st ed. New Delhi: Ministry of Health and Family Welfare, Department of Indian Systems of Medicines and Homeopathy, 1996, A53-A55.
14. WHO. Quality control for medicinal plant material. New Delhi: AITBS Publishers, 1998, 46.
15. Kokoski J, Kokoski R and Salma FJ. Flourescence of powdered vegetable drugs under ultraviolet radiation. J am Pharm Assoc. 1958; 47:715-717.
16. Chase CR and Pratt RJ. Flourescence of powdered vegetable drugs with particular reference to development of a system of identification. J Am Pharm Assoc. 1949; 38:324-333.
17. Khandelwal KR. Practical Pharmacognosy, Techniques and Experiments. 27th ed. Nirali Prakashan, 2016.
18. WHO. Macroscopic and microscopic Examination: Quality Control Methods for Medicinal Plant Materials. Vol. 9. Geneva: WHO, 1998, 22-4, 33.
19. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 37th ed. Nirali Prakashan, 2007.
20. S. Rakesh, N. Dheeraj, N. Sanjay. 'HPTLC' an important tool in standardization of herbal medical product: A review. J. of scientific and innovative research. 2013; 2 (6):1086-1096.
21. WHO. Guidelines for the Assessment of Herbal Medicine. Munich: WHO, 1991.
22. Rabari H, Pandya S, Vidyasagar G, Gajra B. Pharmacognostical and phytochemical investigation of *Cocculus pendulus* (J.R. and G. FORST.) Diels Leaf. Int J Pharm Biol Sci. 2010; 1:1-13.
23. K Tarun. Standardization of Herbal drugs- A review. Int. J. of Uni. Ph. and Biosc. 2013; 2(4):7-18.
24. MB Wickramasinghe. Quality Control, Screening, Toxicity, and Regulation of Herbal Drugs. Wiley online library, 2006, 25-57.
25. Aiswarya KP, Sruthy Unnikrishnan N, Mahesh S, Laija S Nair. Phytochemical analysis of leaf, bark and fruit extracts of *Baccaurea courtallensis* Muell. Arg. Journal of Pharmacognosy and Phytochemistry, 2016, 196-198.