Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 3, 2012

Research Article

PHARMACOGNOSTIC AND PRELIMINARY PHYTOCHEMICAL INVESTIGATIONS OF ANACARDIUM *OCCIDENTALE (*LINN.) LEAVES

YOGINI JAISWAL[1], VINAYAK NAIK [3], PRATIMA TATKE[1*], SATISH GABHE[1], ASHOK VAIDYA[2]

[1]C.U.Shah College of Pharmacy, S.N.D.T Women's University, Mumbai- 400049, [2]ICMR Advanced Centre of Reverse Pharmacology in Traditional Medicine, Kasturba Health Society, Vile Parle-(W), Mumbai- 400 056, [3]Piramal Life Sciences Limited, 1, Nirlon Complex, Goregaon (East), Mumbai - 400 063, India. * Email: patatke@gmail.com

Received: 16 Mar 2012, Revised and Accepted: 25 Apr 2012

ABSTRACT

The present paper attempts to evaluate the physicochemical and preliminary phytochemical characteristics of Anacardium occidentale (Linn.), belonging to family Anacardiaceae. Anacardium occidentale is a tree native to Brazil and also found in tropical countries like Malaysia and India. The plant is reported to have anti-fungal, antioxidant and anti-inflammatory activities. However there are no reports for the detailed standardization of leaves. Hence a detailed study of the phytochemical and pharmacognostical parameters has been carried out. The macroscopic, microscopic characteristics of Cashew leaves were identified and physicochemical parameters were studied. Phytochemical tests reveal the presence of carbohydrates, proteins, saponin glycosides, flavonoids, alkaloids, tannins and phenolic compounds in ethanol and aqueous extract of leaves. TLC analysis was performed in order to obtain a fingerprint and identify various phytoconstituents present in the extracts. TLC of the ethanol extract was developed in the mobile phase of toluene:ethyl acetate:MeOH:formic acid (6:6:1:0.1v/v/v/v) and derivatisation with $FeCl_3$ solution revealed the presence of tannins and phenols which gave a bluish black coloration.

The study reveals specific identification characteristics for the particular crude drug which will be of significant use in identification and control to adulterations of the raw drug and can serve as a reference for any further investigations.

Keywords: Anacardium *Occidentale,* Anacardiaceae.

INTRODUCTION

The Ayurvedic system of medicine has been established in India since a number of decades, and it still remains the foundation of the traditional system of Indian medicine. Since past few decades a number of Ayurvedic and other herbal products have gained importance globally and have achieved a great demand.

With an increasing commercialization of herbal products, there was a need for the scientific community to introduce a quality control system for plant based medicines. The Government of India introduced an amendment in 1964 to the Drug and Cosmetics Act 1940, to have a stringent quality control on Ayurvedic, Siddha and Unani drugs. Progressively, the development of standards for the identity, purity and strength of single drugs and formulations assumed importance for the effective enforcement of the provisions of the Act. The raw materials in herbal medicines are expected to conform to uniform standards. It is necessary to identify the drugs, determine their quality and to detect adulterations [1]. Cashew is a tropical tree native to Brazil, and it belongs to the family Anacardiaceae. Nevertheless, now it is also widely grown in other tropical countries like India. Standards for quality control of herbal formulations or preparations are based on pharmacognostical, physicochemical, phytochemical and biological parameters. Anacardium occidentale (Cashew) has been explored for varied pharmacological and phytochemical investigations, however there are no reports published for the standardization of cashew [2-4].

Hence, an attempt was made to carry out the preliminary phytochemical screening and pharmacognostical evaluation of leaves of Cashew that can serve as a reference material for any studies related to adulteration and pharmacognostical investigations.

MATERIALS AND METHODS

Plant Material collection and authentication

Leaves were collected from Tungareshwar forests of Vasai Taluka, Dist. Thane in the state of Maharashtra, India. The plant specimen was authenticated at the Botanical Survey of India, Pune; (M.S). A herbarium of the plant specimen (specimen voucher number no. YOGA1/No.BSI/WC/Tech/2008/69) was submitted at the Botany Department of BSI, Pune.

Pharmacognostical studies

Organoleptic characterization

In order to determine the organoleptic characters of the drug, the color, odor and taste of the plant material were estimated by visual and sensory evaluation.

Macroscopy

The following macroscopic characters for the fresh leaves were noted: size and shape, color, odor and taste**,** base, tip, venation, margin, arrangement and texture [5-6].

Microscopic analysis and powder characteristics

Transverse section and Microscopic characters

The paraffin embedded specimens were sectioned using a Rotary Microtome. The thickness of the prepared sections was about 10 - 12µm. Dewaxing of the sections was done by routine procedure[7]. Glycerine mounted temporary slide preparations were made for the cleared materials. Powdered materials of leaves were cleared with NaOH and mounted in glycerine medium after staining. Different cell components were then studied and measured.

Microscopic assessment was carried at a projection of 10X. The presence/ absence of epidermal cells, covering trichomes, xylem, phloem, stomata and collenchyma were observed. The transverse sections of cashew leaves through the lamina and the midrib region were observed [8].

Photographs of 10X magnifications were taken with Nikon lab photo 2 microscopic units and Motic software. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed.

Examination of powder characteristics

Estimation of the powdered drug for starch grains, lignified cells and calcium oxalate crystals were carried out using the method reported by Sailor *et al* [9]. The leaves were dried for 4-6 hrs and powdered using electric grinder and the powder was passed through sieve no. 60. Powder (size sieve no. 60) of the dried leaves was used for the study of powder microscopic characters. The powdered drug was treated with phloroglucinol, glycerin and iodine solution[10].

Physicochemical parameters

The various physicochemical parameters were studied by the method adopted by Sailor *et al.* The parameters included determination of total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value, ether soluble extractive value and moisture content[11].

Preliminary phytochemical screening

One gram of the extract of leaves of cashew was dissolved in 100 ml of respective solvents used for extraction to obtain a stock of concentration 1% (v/v). The extracts thus obtained were subjected to preliminary phytochemical screening [1, 12].

TLC analysis

TLC analysis helps in identifying various constituents of a sample. Thus, a suitable mobile phase was developed and other chromatographic parameters were optimized for extracts of leaves of cashew. Derivatisation of the developed plates was carried out in order to estimate the presence of various phytoconstituents in the extracts.

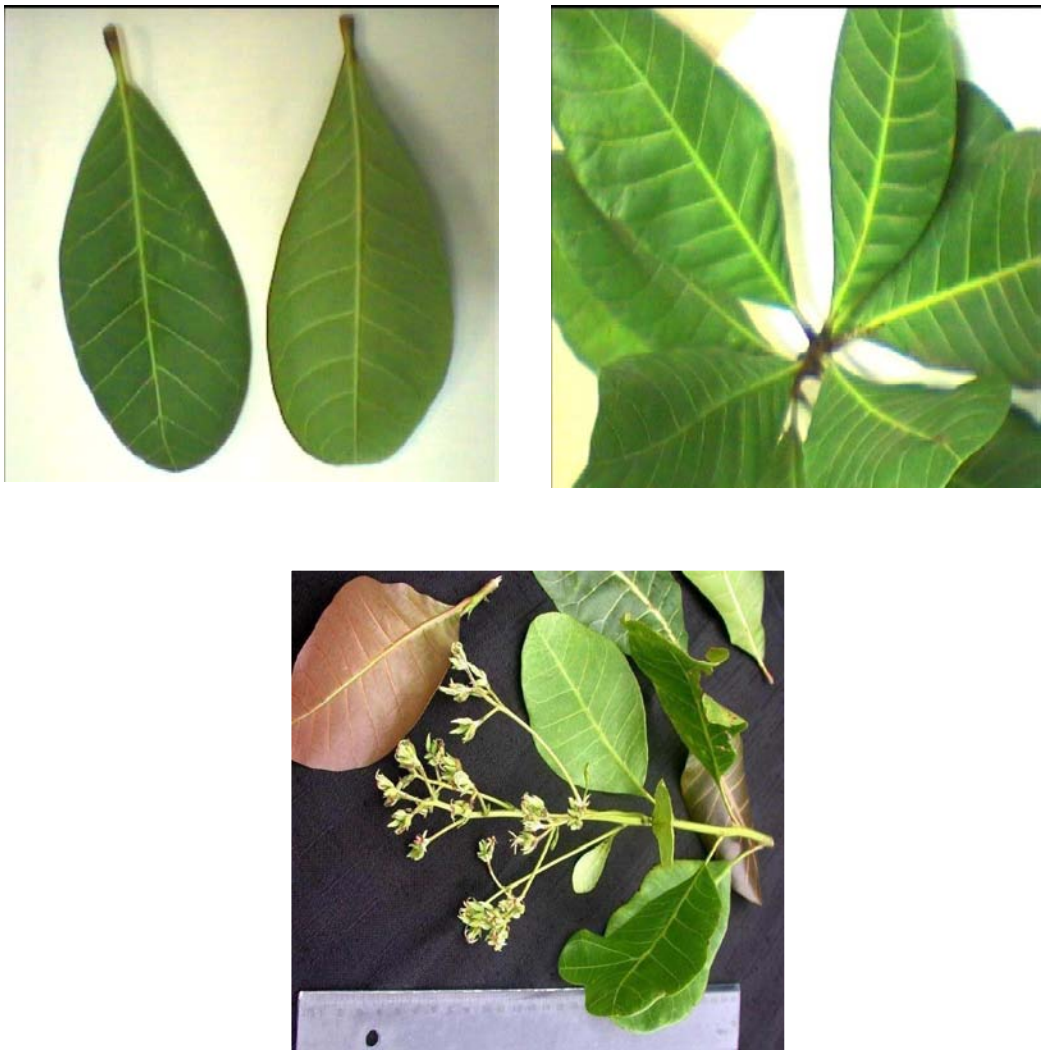
RESULTS AND DISCUSSION

Organoleptic characters

The powder of dried cashew leaves is green in color, with no characteristic odor and the taste is slightly astringent.

Macroscopic characteristics

The macroscopic study of cashew leaves is depicted in **Fig. 1.** The leaves were observed to be petiolated , elliptic obovate in shape, 4 to 22 cm long and 2 to 15 cm broad, a cuneate base, with obtuse tip, reticulate venation, entire and smooth margin, a spiral arrangement and leathery texture.



(a) Leaves of cashew(b) Twig of cashew

(c) Stem of cashew

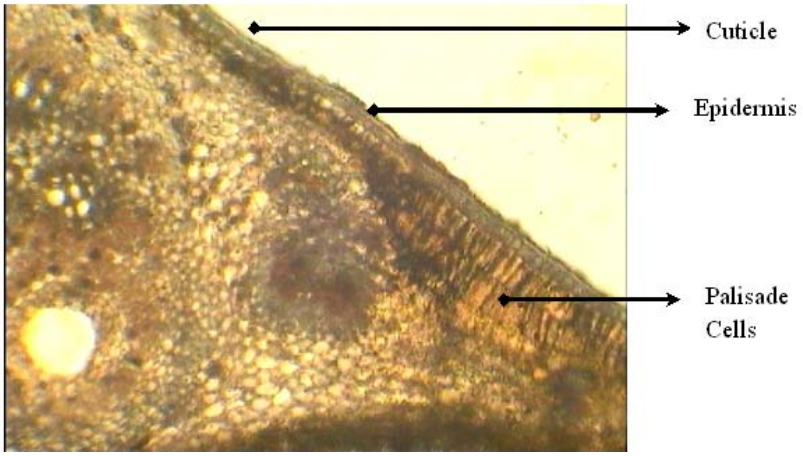
Fig. 1: Macroscopic characters of *Anacardium occidentale* leaves

Microscopic characteristics

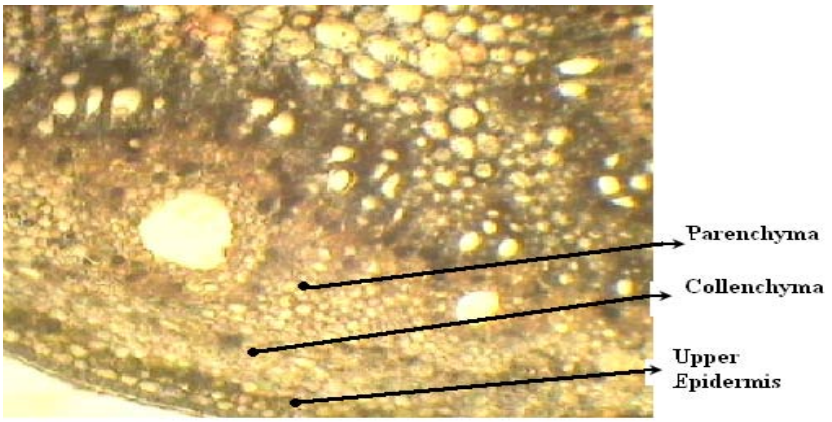
Study of transverse section of Leaf through midrib

As observed in **Fig. 2,** and **Fig. 3**, the upper epidermis consisted of a single layer of barrel-shaped cells. The epidermal cells were covered by a thick cuticle and stomata were found along the epidermis. The mesophyll consisted of two to three layers of compact cylindrical palisade cells and 4-5 layers of parenchyma. In the midrib region; the upper epidermis was distinct, followed by few layers of collenchymas and a wide region of different sizes of parenchyma cells. The vascular bundle region was covered by endodermis and most of the part of midrib was filled

with corticle parenchyma and lignified xylem. Each vascular bundle protected by an upper and a lower patch of sclerenchyma cells. A wide nonlignified phloem region was found towards the lower epidermis protected by thick sclerenchyma cells. The xylem was formed of vessels arranged into 5-8 rows of vessels, in each row there were 2-6 vessels. The parenchyma cells below the vascular bundle were formed of 3-5 layers varying sizes of cells.



(a)



(b)

Fig. 2: T.S of lamina of cashew leaf

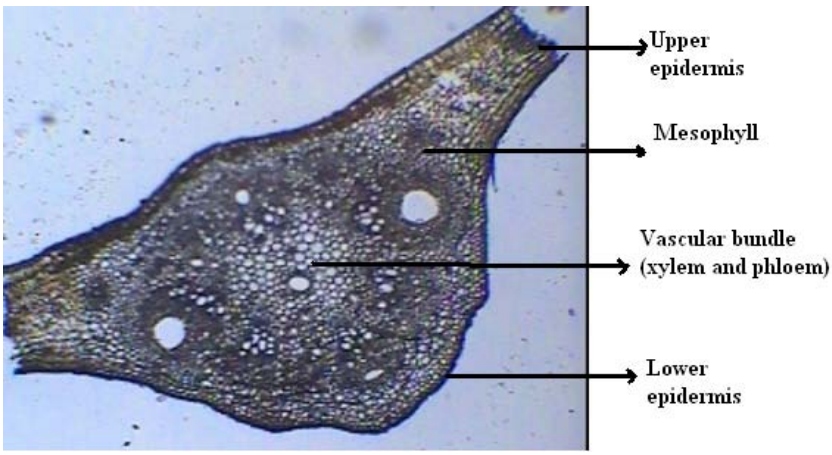


Fig. 3: T.S. of cashew leaf through midrib

Study of transverse section of petiole

The general structure of the transverse section of the petiole appeared circular. The outermost layer is formed of one layer of epidermis with no hairy structures. The vascular bundles are arranged in a circle, and each vascular bundle is preceded by pericyclic fibers. The phloem region is formed of primary and secondary phloem and they are followed by the xylem. The pith is a wide region of thickened parenchyma cells **(Fig. 4)**.

Study of diagnostic characters (powder characteristics) of leaves

The diagnostic characters revealed in study of powder of cashew leaves were epidermal cells, stomata, palisade cells and trichomes as

seen in **Fig.5**. The trichomes were single celled covering trichomes with sharp ends. Some collapsed trichomes were also observed. Epidermal cells with ranunculaceous stomata. Stomata were surrounded by subsidiary cells, resembling other epidermal cells. Epidermal cells are polygonal with irregular celled stomata. The palisade cells, parenchyma with epidermal cells resemble the lamina portion of the leaves. The stomata were paracytic, rubiaceous celled with irregular subsidiary cells.

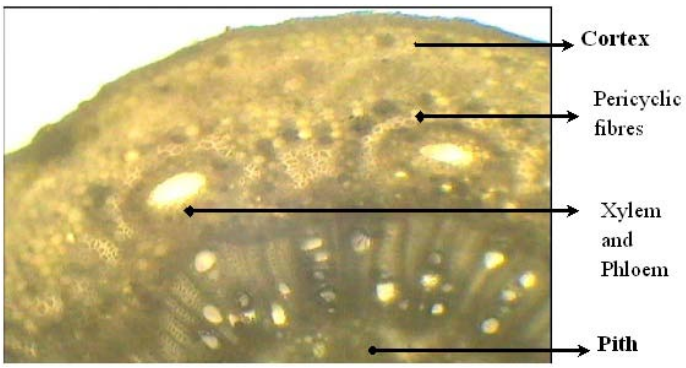
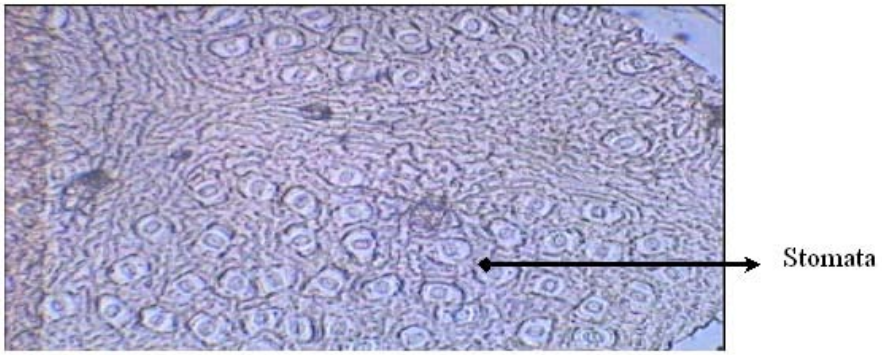
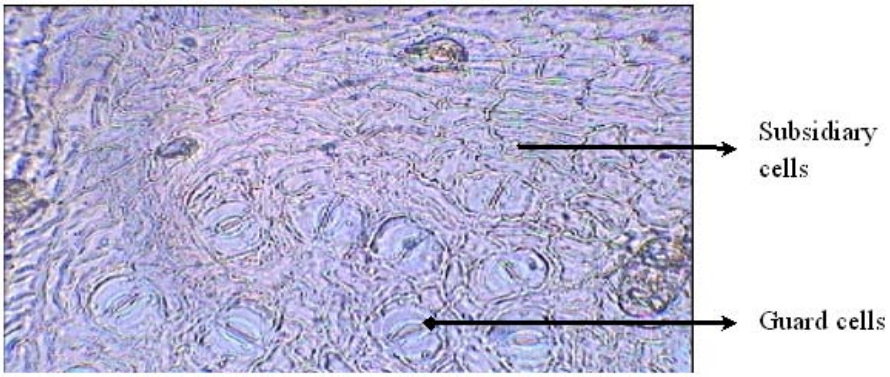


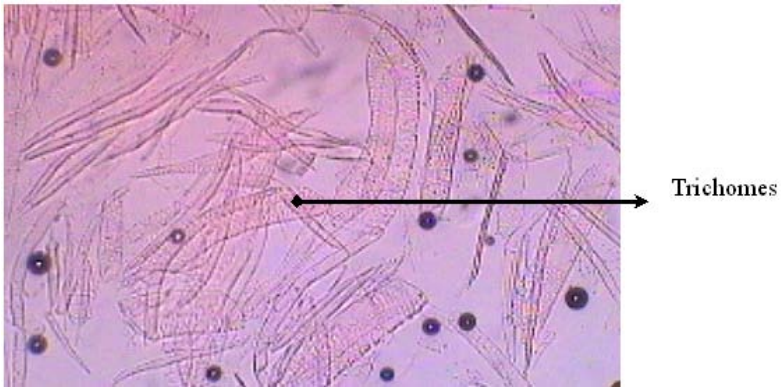
Fig. 4: T.S. of petiole of cashew leaf



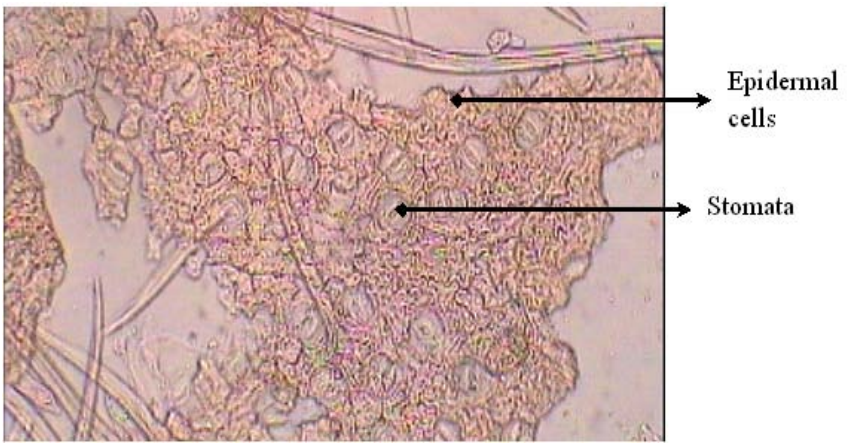
(a) Stomata of cashew leaf



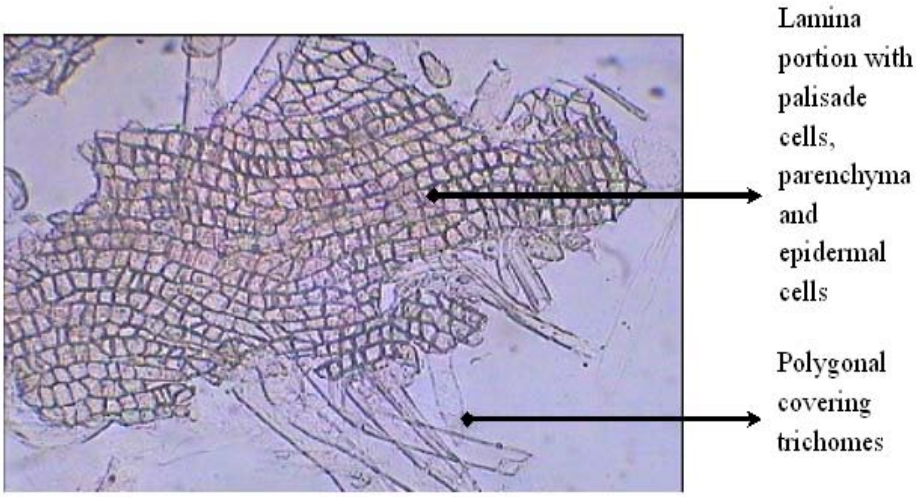
(b) Stomata of cashew leaf



(c) Trichomes of cashew leaf



(d) Epidermal cells of cashew leaf



(e) Palisade cells of cashew leaf

Fig. 5: Powder characteristics of leaves of cashew

Physicochemical parameters

The *total ash* method estimates the total amount of material that remains after ignition and the amount of heavy metals and inorganic compounds and it includes both the "physiological and non-physiological" ash, which is the remainder of the extraneous matter (e.g. sand and soil) that adheres to the plant surface. The acid insoluble ash comprises mainly of silica and is a sign of contamination with earthy material. The water soluble ash determines the amount of inorganic elements present in drugs.

Table 1: Determination of ash values

Type of ash	Leaves of cashew (%) ± SEM
Total ash	10.5 ± 0.2
Acid insoluble ash	1.0 ± 0.5
Water soluble ash	4.5 ± 0.4
Sulphated ash	2.4 ± 0.3

n=3 determinations for values of each test mentioned above

Table 2: Determination of loss on drying

Leaves of cashew (% w/w) ± SEM
Loss on drying 7.5 ± 0.7

n=3 determinations for values of the test mentioned above

Table 3: Determination of various extractive values

Extract	Leaves of cashew (%) ± SEM
Alcohol soluble extractive	20.9 ± 0.9
Water soluble extractive	7.6 ± 0.5
Ether soluble extractive	3.5 ± 0.3

n=3 determinations for values mentioned above

Table 4: Determination of pH values

	Extract of cashew leaves ± SEM
pH values	5.5 ± 0.1

n=3 determinations for values mentioned above

An excess of water in plant material encourages microbial growth, the presence of fungi or insects, and deteriorates the phytoconstituents followed by hydrolysis. Limits for moisture content are therefore set for every given plant material. The test for *loss on drying* estimates both water and volatile matter. Determination of *extractive values* gives an estimation of the amount of active constituents extracted with solvents from a specific amount of plant material. Various physicochemical parameters of powdered drug has been investigated and reported in **Table 1 - 4**.

Preliminary phytochemical screening

All the prepared extracts were subjected to qualitative chemical test and results are indicated in **Table 5**. The result shows that ethanol and aqueous extract of leaves contain carbohydrates, proteins, saponin glycosides, flavonoids, alkaloids, tannins and phenolic compounds. Both the extracts have the presence of similar constituents. Gums, mucilage, amino acids, and organic acids were found to be absent in leaves of cashew. The extraction for leaves was carried out with solvents of almost similar polarity. Hence, phytoconstituents of similar nature were found in extracts of leaves.

Development and optimization of TLC parameters

Thin layer chromatographic technique was used to separate the constituents present in the drug. The optimized mobile phase was developed by trying various solvent systems to separate the maximum number of constituents in the drug. TLC of the ethanol extract was developed in the mobile phase of toluene: ethyl acetate: MeOH: formic acid (6:6:1:0.1v/v/v/v) **(Fig. 6)**. After development the plate was visualized in UV 254 and 366 nm. The plate was then derivatised with $FeCl_3$ solution to confirm the presence of tannins and phenols which gave a bluish black coloration.

Table 5: Results of qualitative chemical tests

S. No.	Chemical Tests	Ethanol extract	Aqueous extract
1	**Test for Carbohydrates**		
	Molisch's test	+	+
	Benedict's test	+	+
	Test for Non-reducing sugars	+	+
2	**Tests for Proteins**		
	Millons test	+	+
3	**Tests for Amino Acids**		
	Ninhydrin test	-	-
4	**Test for Fats and Fixed Oil**	-	-
	Stain test		
5	**Saponification test**	+	+
6	**Test for Sterols and Triterpenoids**		
	Libermann- Buchard test	-	-
7	**Test for Glycosides**		
	Legal's test for Cardiac Glycosides	-	-
	Keller Killiani test [for Deoxy sugars]	-	-
	Froth Test for Saponin Glycosides	+	+
8	**Test for Flavonoids**		
	Shinoda test (Magnesium Hydrochloride reduction)	+	+
	Alkaline reagent test for Flavonoids	+	+
9	**Tests for Alkaloids**		
	Dragendorff's test	+	+
10	**Test for Tannins and Phenolic Compounds**		
	Ferric chloride test	+	+
11	**Tests for organic acids**	-	-
	Calcium chloride test		

*The symbol **(+)** denotes presence and **(-)** denotes absence of phytoconstituents.*

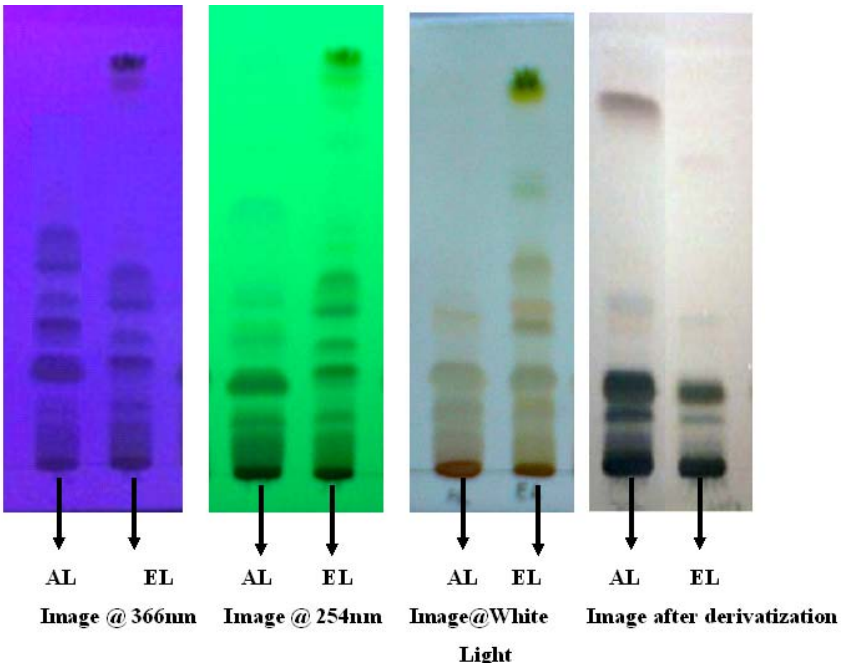


Fig. 6: HPTLC Fingerprints of extracts of leaves of cashew

AL- aqueous extract and EL- ethanol extract of leaves

CONCLUSION

Evaluation of macroscopic, microscopic and physicochemical characteristics of *Anacardium occidentale* leaves was carried out. The results and observations can provide evidences for establishing the identity of the leaves and can serve as routine quality control tests for monitoring the quality of the leaves powder.

ACKNOWLEDGEMENT

The authors are thankful to the Indian Council of Medical Research (ICMR), India for funding this project.

REFERENCES

1. Harbone JB. Phytochemical Methods – A Guide to Modern Techniques of Plant Analysis. 3rd ed. London UK: Chapman and Hall publishers; 1998.
2. Abas F, Lajis N, Israf D. Antioxidant and nitric oxide inhibition activities of selected Malay traditional vegetables. Food Chem. 2006; 95: 566–73.
3. Kudi A, Uhoh J, Eduvie L. Screening of some Nigerian medicinal plants for antibacterial activity. J. Ethnopharmacol. 1999; 67: 225-28.
4. Goncalves J, Lopes S, Oliveira D. *In vitro* anti-rotavirus activity of some medicinal plants used in Brazil against diarrhea. J. Ethnopharmacol. 2005: 99(3); 403–07.
5. Trease GE and Evans WC. Pharmacognosy. 15th ed. London: WB Saunders; 2002.
6. Wallis TE. Textbook of Pharmacognosy. India: SK Jain Publishers; 1985.
7. Johansen DA. Plant Microtechnique. New York: McGraw Hill Book Co.; 1940.
8. African Pharmacopoeia. General methods for Analysis, O.A.U./ST Scientific Publications Lagos. 1st ed. 1986; 2: 223-37.
9. Sailor GU, Ghanshyam P, Ashvin VD, Seth NR, Seth AK. Pharmacognostical and Preliminary Phytochemical Investigation of *Leucas cephalotes* (Roth) Spreng. Int. J. Pharm. Res. 2010; 2:14 -21.
10. Kim NJ. Evaluation of some herbal drugs – Systematic approach Fitoterap.1982; 59(6):494 - 95.
11. Sailor GU, Ghanshyam P, Ashvin VD, Seth NR, Seth AK. Pharmacognostical and Preliminary Phytochemical Investigation of *Leucas cephalotes* (Roth) Spreng. Int. J. Pharm. Res. 2010; 2:14-21.
12. Khandelwal KR. Practical Pharmacognosy: Techniques and Experiments. India: Nirali Prakashan. 1999; 146-48.
13. Blessy BM, Suresh KJ. Phytochemical Analysis of *Citrus Limonum* Pulp and Peel. Int. J. Pharm. Pharmaceut. Sci. 2012; 4(2), 369-71.
14. Arumugam KL, and Venugopal SA. Phytochemical analysis and antioxidant activity of *Barringtonia acutangula* (l.) Gaertn. Leaves. Int. J. Pharm. Pharmaceut. Sci. 2012; 4(2), 277-81.