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Research Article

AUTHENTICATION AND QUALITY EVALUATION OF AN IMPORTANT AYURVEDIC DRUG AVERRHOA CARAMBOLA LINN LEAVES

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ABSTRACT

Objective: To evaluate the pharmacognostical and physico-chemical characters of *Averrhoa carambola* Linn. an important medicinal plant in the Indian system of medicine.

Methods: The pharmacognostic studies out in terms of various investigations like organoleptic or morphological characters, microscopical or anatomical studies, physico-chemical evaluations (loss on drying, ash values, extractive values), preliminary phytochemical screening, TLC finger print profiling and fluorescence analysis of powdered crude drug as per WHO recommended guidelines for standardizations.

Results: Macroscopically, the leaves are observed to be compound, pinnate, leaflets are ovate to ovate lanceolate in shape and soft. It shows glabrous surface, acute apex, entire margin and oblique base. Microscopically, the leaf showed the presence of single layered epidermis covered with striated cuticle. Some of the epidermal cells elongate to form covering trichomes. The characteristic microscopic features of leaves were observed as trichomes, multicellular trichomes, xylem cells, phloem cells, collenchyma and vascular bundles. Physiochemical parameters such as percentage of foreign matters, ash values, loss on drying, swelling index extractive values were determined. Preliminary phytochemical screening showed the presence of carbohydrates, terpenoids, glycosides, Flavonoids and phenolic compounds.

Conclusions: The results of the study can serve as a valuable source of information and provide suitable standards for identification of this plant material in future investigations and applications. These information will also be helpful to differentiate *A. carambola* L from the closely related other species.

Keywords: Averrhoa carambola, pharmacognostical, Leaves, quality control.

INTRODUCTION

Diseases have been known for thousands of years and many ingenious methods have been followed for the relief of mankind. On entering into the 21st century, we have subjected ourselves to such a susceptible environment that it has almost become impossible for our natural immune system to give us the fair share of protection. Almost every source of life and matter surrounding human being has been used in some form or other to treat diseases. Since the beginning of the history of mankind plants have been used for the treatment of various diseases [1].

Today, several medicinal plants and their products are still in use, being employed as home remedies, over the counter drugs as well as raw materials for the pharmaceutical industry and they represent a substantial proportion of the global drug market [2]. However a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and stringent quality control. There is a need for documentation of research work carried out on traditional medicines [3]. Therefore it has become extremely important to make an effort towards standardization of the plant material to be used as drug.

Averrhoa carambola L. belonging to family Oxalidaceae is commonly known as 'Kamrakh', Carambola apple or 'Star fruit' had attained the status of a popular commercial crop in the India [4]. It is edible and has numerous uses. The ripe fruit may be processed into fermented or unfermented drinks, jam or jelly, can be eaten fresh or as dessert. The unripe fruit may also be eaten as a vegetable. The sweet type is processed into wine in Surinam [5].

Averrhoa carambola L. is traditionally found to be useful for many ailments. Plant is a small, handsome evergreen tree about 9.0 m in height with close dropping branches. The leaves are antipruritic, antipyretic, anthelmintic and are also useful in scabies, fractured bones and various types of poisoning as an antidote, intermittent fevers and elimination of intestinal worms [6-8]. Even though this plant has gained scientific importance, recently, there is a need for the pharmacognostic standardization. Hence the present study highlights the pharmacognostical as well as phytochemical studies including parameters such as macroscopic, microscopic characters, physiochemical evaluation (loss on drying, ash values, extractive values) and preliminary phytochemical studies of the leaves. TLC finger print profiling and fluorescence analysis of powdered crude drug were carried out and the salient qualitative and quantitative parameters were reported. Star fruit and has been used extensively for treatment of some diseases like inflammation [9], Hypotensive [10], hepatotoxicity [11] and antioxidant [12]. Though the plant has been reported for many biological activities, no scientific data available to identify the genuine sample. The present investigation of *Averroha carambola L.*is therefore taken up to establish quality profile of the leaves which will help in crude drug identification as well as in standardization.

MATERIALS AND METHODS

Collection and authentication

The fresh leaves of commonly growing tree *Averroha carambola* was collected from the field area of Nanpara district Bahraich, U.P. India. For identification and Taxonomic authentication, sample of plant material was given to National Botanical Research Institute (NBRI) Lucknow, India. The plant material was confirmed and authenticated by Dr. A. K. S. Rawat, Scientist and Head, Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute Lucknow, India with report no. CIF-RB-2-132.

The fresh leaves were used for the study of macroscopic and microscopical characters. Whereas collected plant materials were shade-dried and coarsely powdered. This coarse powder was used for the determination of ash values, extractive values, and preliminary phytochemical investigation was studied as per standard methods.

Chemicals and reagents

All the chemicals and reagents used were of laboratory grade.

Extraction of plant materials

100 gm coarse powder of air dried leaves of *Averroha carambola* L. were packed in muslin cloth and subjected to soxhlet extractor for continuous hot extraction with distilled water, ethanol, chloroform and petroleum ether separately. Then the each extracts were filtered and filtrate was evaporated to dryness. The percentage yield of the water, ethanol, petroleum ether and chloroform extracts was 13.41%, 9.80 %, 1.2 % and 1.4 % respectively.

Macroscopic and microscopic studies

The macromorphology of the leaves were studied according to standard methods [13-14]. For anatomical studies hand section of leaves was taken, stained and mounted following usual microtechniques [15] and representative diagrams were taken with the help of inverted microscope for photo documentation (Leitz, Japan).

Physicochemical analysis

Physicochemical analysis i.e. alcohol (90 % ethanol) and water soluble extractive values, fluorescent analysis [16, 17], total ash, acid-insoluble ash, water-soluble ash, swelling index and foreign matter [18]. Calibrated digital pH meter was used to measure the pH of 1% and 10% aqueous extracts and also loss on drying was noted.

Preliminary phytochemical screening

Preliminary phytochemical screening was done for the detection of various classes of chemical constituents by using standard procedures described by Harborne [19] and Khandelwal [20].

Thin layer chromatography (TLC)

Thin layer chromatography studies of the ethanol and aqueous extracts carried out in various solvents using Silica gel G as adsorbent and the R_f values were determined [21].

RESULTS

Macroscopic characters

The leaves of *Averrhoa carambola* were observed to be compound, pinnate, 15-25 cm long and the 3.8–7 cm long leaflets which are 5 to 11 nearly opposite in manner. Leaflets are ovate to ovate lanceolate in shape and soft. The top sides of the leaves are smooth and bright-green. The undersides are finely hairy and pale green in colour. The leaflets tend to fold together at night. They are also sensitive to abrupt shock. The leaves are delicate and attractive as well. It shows glabrous surface, acute apex, entire margin and oblique base with characteristic odour and taste (Figure 1, Figure 2).

Fig. 1: *Averrhoa carambola*-a. Tree; b. Leaves; c. Flower; d. Fruit

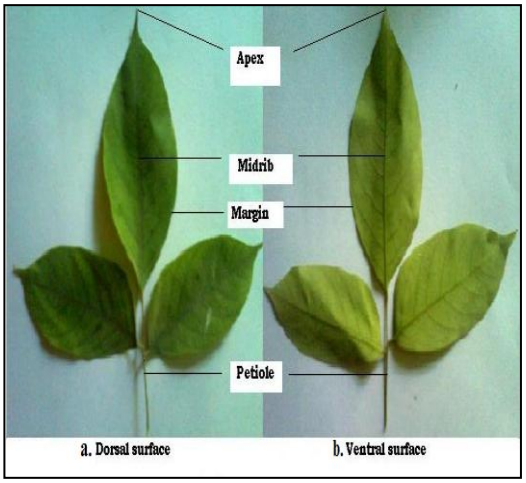

Fig 2: Morphology of leaf

Microscopical characters

TS of leaf through Midrib

The T.S. of midrib shows single layered epidermis covered with striated cuticle. Epidermal cells are more or less round to oval in shape. Some of the epidermal cells elongate to form covering trichomes which are 3-4 cells long and show mostly pointed end. Beneath the epidermal cells the layers of collenchymatous cells were present. The parenchymatous cells are mostly round or oval in shape having bigger space. The central region of the mid-rib is occupied by vascular tissues which are arch shape showing, phloem, capping the xylem. The phloem consist sieve-tubes, compaining cell and phloem parenchyma. The xylem consists of vessels, tracheids and xylem parenchyma which show distinctly thick wall lignification and wide lumen. Below the arch of the xylem is a wide region of parenchymatous cell which also appear similar to parenchymatous cells described earlier (Figure 3).

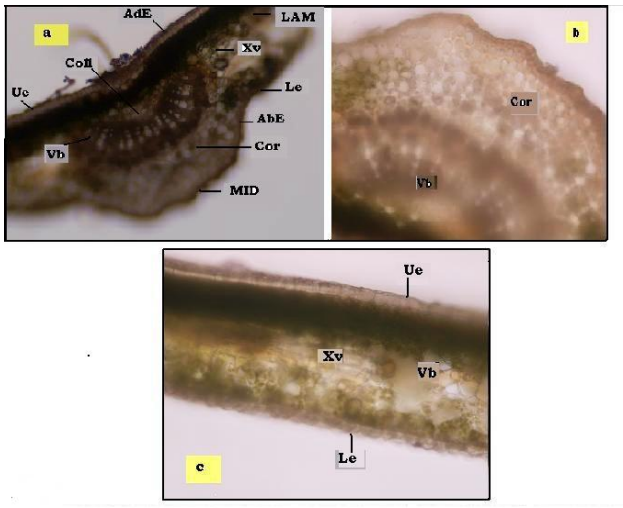

Fig. 3: Microscopical character of leaves.

a. TS of mid rib with lamina; b. region of mid rib from abaxial epidermis to vascular bundle enlarged; c. region of lamina (Abbreviation: AdE- adaxial epidermis; AbE- abaxial epidermis; LAM- lamina; MID – midrib; Vb- vascular bundle; COL-collenchyma; COR- cortex; Xv-xylem vessels; Le- lower epidermis; Ue- upper epidermis

TS of leaf through Petiole

Transverse section of petiole shows single layered epidermis, consisting of flattened, elongated cells with covering of cuticle.

Under the epidermis 2-5 layered collenchymatous and 2-6 layered circular, thin walled, chlorenchymatous cells with intracellular spaces were observed. Vascular bundles were arranged in a single ring. Some bundles were capped by one or two layered, thick walled, lignified, polygonal pericyclic sclerenchyma. In centre very wide pith was observed which was composed of large parenchymatous cells (Figure 4).

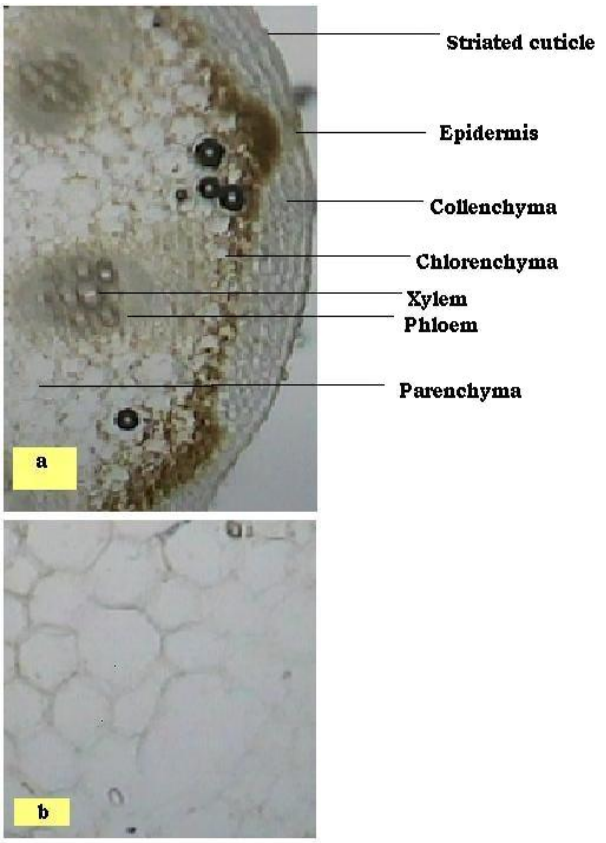

Fig. 4: (a) T.S. of petiole showing cuticle, collenchymatous cells, xylem, phloem and epidermis; (b) T.S. of petiole showing parenchymatous cells in high magnification.

TS of leaf through stem

Transverse section of the stem shows covering trichomes, uniseriate and spaced. Epidermis is uniseriate and covered with thin cuticle. The epidermal cell is rectangular to square. Cell wall is moderately thickened. Periderm consists of many layers; its cells are rectangular to square. The cortex consists of collenchyma and polygonal parenchyma. Endodermis is made up of one layer. Medullary ray is present. Pericycle comprises of polygonal cells and small cells of parenchyma. The phloem cells are arranged in groups and present in form of strands. Xylem appears in form of continuous cylinder. Intra-xylary ploem is present in form of strand and at the periphery of the pith. The pith is unlignified and composed of polygonal parenchyma (Figure 5).

Physicochemical parameters

The physico-chemical characters of powder drug of leaves of *A.carambola* such as total alcohol soluble extractive, water soluble extractive, ash value, acid insoluble ash, water-soluble ash, loss on drying, swelling index, and foreign matter are presented in Table 1. The fluorescence analysis of the powder drug of *A.carambola* in various solvents and chemical reagents was performed under normal and Ultra Violet (UV) light Table 2. The pH of 1% solution was 6.2 and pH of 10% solution of powered drugs of *A.carambola* was 5.6.

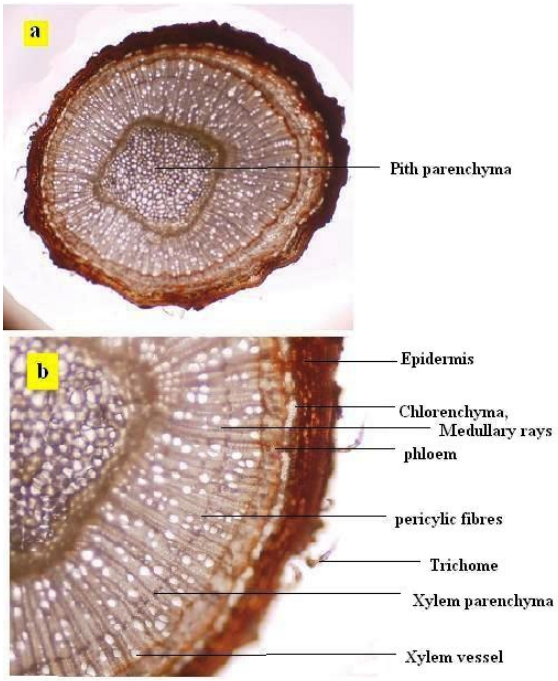

Fig. 5: T.S. of stem of Star Fruit

Table 1: Physicochemical parameters of *A.carambola* leaves

Quantitative parameter	Values obtained (%) w/w
Alcohol soluble extractive	12.2
Water soluble extractive	16.6
Total ash	8.5
Acid insoluble ash	1.7
Water – soluble ash	4.0
Loss on drying	11.3
Swelling index	2.0
Foreign matter	1.2

Table 2: Fluorescence analysis of *A.carambola* leaves

Solvent used	Day Light	U V light (254nm)	U V light (366nm)
Powder as such	Dark green	Slight green	Blackish green
1N HCl	Greenish brown	Brownish green	Dark green
50% HCl	Light green	Green colour	Dark green
50%HNO_3	Green	Brownish green	Bluish green
50%H_2SO_4	Slightly green	Brownish green	Light green
1N NaOH	Light yellow	Green colour	Moderate green
Alcoholic NaOH	Light green	Green colour	Dark green
Methanol	Light green	Dark green	Dark green
Benzene	Slightly Yellow green	Florescent green	Reddish brown
$FeCl_3$	Brownish green	Light green	Green

1% KOH	Blackish green	Light green	Dark green
Lead acetate	White	Florescent white	White
Distilled water	Light green	Pale Green	Dark brown

Preliminary phytochemical screening

The preliminary phytochemical investigation of the leaves extract of *A.carambola* L. showed the presence of phytosterols, flavonoids, Acidic compound, carbohydrates, glycosides and alkaloids in table 3. The result of powdered drug reaction with different reagent was shown in table 4. The fluorescence analysis of the powdered drug from the *A.carambola* leaves in various solvents was performed under normal and UV light. All the leaves extracts are examined in short UV (254nm) and long UV (366 nm) to detect the fluorescent compounds. The observations are given in Table 3.

Table 3: Qualitative phytochemical analysis of *A.carambola* leaves extract.

S.No.	Constituents	*A.carambola leaves*
1	Alkaloids	+
2	Carbohydrate	+
3	Glycoside	+
4	Phenolic compound and tannins	+
5	Flavonoides	+
6	Proteins and Amino Acids	-
7	Saponins	-
8	Sterols	+
9	Acidic compound	+
10	Lipids/ fats	–

Table 4: Powdered drug reaction with different reagent

Treatment	Observation
Conc. HCL	Dark green
Conc. HNO3	Light brown with whitish foam
Conc. H2SO4	Greenish yellow
Glacial Acetic acid	Light yellow
Iodine solution	Dark brown
NaoH in Methanol	Light green

TLC profile of *A.carambola leaves*

Thin layer chromatography of the aqueous and ethanolic extracts was carried out separately using n-butanol: Acetic acid: water (2:4:6) for aqueous extract and ethanolic extract as mobile phase and the R_f values were recorded in Table 5. The visualizing reagent employed was anisaldehyde-sulphuric acid reagent to effect visualization of the resolved spots. TLC finger printing studies on ethanol extract showed presence of various phytoconstituents with their respective R_f values.

Table 5: Thin layer chromatography of leaf extracts.

S.no.	Leaves extracts	Solvent system	Number of spots	R_f value
1.	Aqueous extract	n-butanol : Acetic acid : water (2:4:6)	3	0.12, 0.40, 0.72
2.	Ethanol extract	n-butanol : Acetic acid : water (2:4:6)	4	0.26, 0.54, 0.73, 0.91

Standardization is an essential measure of quality, purity and authenticity. As a part of standardization study, the macroscopical examination of *A.carambola* leaves was studied. As there is no pharmacognostic work recorded on this medicinally potent plant, the present work was undertaken to lay down the standards which could be useful for establishing its authenticity. Macro and micro standards are useful identifying parameters for authentication of the drug. Macroscopical evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of drugs. The macroscopical characters of the leaves of plant can serve as diagnostic parameters. The extractive value, ash value, loss on drying and fluorescent analysis of the leaves extracts have been carried out. Percentages of the extractive values were calculated with reference to air-dried drug. The percent extractives in different solvents indicate the quantity and nature of constituents in the extract. The extractive values are also helpful in estimation of specific constituents soluble in particular solvent. The ash value was determined by three different methods, which measured total ash, acid-insoluble ash, and water-soluble ash and the result were tabulated in table. Thin layer chromatography (TLC) is particularly valuable for the preliminary separation and determination of plant constituents. The chromatographic profile may serve as a characteristic finger print for qualitative evaluation of leaves. The information obtained from the preliminary phytochemical screening will reveal the useful findings about chemical nature of the drugs. The plant is rich in secondary metabolites which are of great medicinal value and have been extensively used in the drug and pharmaceutical industry.

CONCLUSION

After present investigation it can be concluded that the standardization and preliminary phytochemical investigation study of *A.carambola* leaves yielded a set of standards that can serve as an important source of information to ascertain the identity, authenticity and to determine the quality and purity of the plant material in future studies. It would also help in distinguishing the plant material of *A.carambola* leaves from its related species. This study is a substantial step and it further requires inevitable long term study to evaluate therapeutic efficacy and toxicity of leaves, to establish as the drug. Further work will emphasize the isolation and characterization of active principles. These studies can also help the ayurvedic manufacturer for the quality, purity, safety and for the selection of the raw material for various formulations.

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