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ORIGINAL ARTICLE **OPEN ACCESS**

Quantitative, Qualitative, Phytochemical Investigations and Antioxidant Activity of *Averrhoa carambola* L. Seed

Swati Singh*, Ashutosh Upadhyay, Anup Kumar Sirbaiya
Department of Pharmacology, NIMS Institute of Pharmacy, Jaipur, Rajasthan, India.
Faculty of Pharmaceutical Chemistry, Maharana Pratap School of Pharmacy, Lucknow, (U.P), India.
For Correspondence: swati.29.singh@gmail.com

ABSTRACT

Chemical diversity in natural goods, such as plant extracts, such as pure chemicals or standardised extracts, provides an endless supply of new therapeutic discoveries. Bioactive chemicals found in medicinal plants are commonly prescribed a wide range of human illnesses and serve a vital part in healing. Plants have the ability to naturally balance the human body's metabolism. Plants have less adverse effects than synthetic drugs, which makes their medical potential stand out. Various parts of plants like root, leaves, bark, seeds, and flowers have various medicinal properties. To our knowledge, no studies have been conducted to look at the phytochemical contents of Averrhoa carambola L. seeds, thus we provide here the results of three separate extracts (Aqueous, Ethyl acetate, Ethanolic, and Petroleum ether) that we took Stepwise pharmacognostical research can help standardise the approach. It is through the results of these investigations that plants can be correctly identified and verified. Herbal medicine's safety and effectiveness are dependent on reproducible quality, which can only be achieved by precise identification and quality assurance of the raw components. It has been discovered that the fruit of Averrhoa carambola L. contains Alkaloids, Glycosides, Protein and Phenol in its preliminary screening. As a result, it's essential to look into the active compounds and examine the pharmacological activity of this plant for therapeutic purposes, as it could be a game-changer in the world of medicine. Total phenol and flavonoid content assessment tests, total antioxidant capacity, and the 1,1-diphenyl-2-picryl-hydrazil (DPPH) free radical assay were used to assess antioxidant activity. Antioxidant activity was evident in a dose-dependent manner with these extracts. The extracts contained phenolic and flavonoid compounds. The current study's findings suggest that the extracts have substantial antioxidant capability. The study's main objective was to discover the plant's use in herbal medication and the chemical components' worth in the dispersion of the investigated plant's seeds.

Keywords: *Medicinal Plants, Herbal medicine, Phytochemicals, Anti-oxidant, Averrhoa carambola L.*

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INTRODUCTION

In developing countries, herbal medicines play a significant role in the provision of health services. The definition of a medicinal plant in ancient Indian literature is extraordinarily broad, with the implication that "All plant parts are major sources of medicinal substances"[1]. Kamrakh (Oxalidaceae) is the traditional name for *Averrhoa carambola* L. (Oxalidaceae), which is also known as star fruit because of its unique shape [2]. The Averrhoa genus contains five species of star-fruit plants, including *A. bilimbi, A. dolichocarpa, A. leucopetala, A. microphylla,* and *A. carambola*, all of which belong to the genus [3]. As the name suggests, the starfruit is an exotic tropical fruit that is sweet and acidic at the same time [4]. Sri Lanka, Indonesia, and India are home to the tropical fruit *Averrhoa carambola* L. The existence of physiologically active substances in this fruit, which provide health advantages and lessen the risk of certain diseases, has sparked rising interest in it all around the world. Polyphenols and ascorbic acid are just two of the plant's many natural antioxidants [5]. Typically, 3 to 5 metres tall, the *Averrhoa carambola* L. tree has a finely fissured light brown bark and leaves that measure 15 to 20 centimetres long. There are five arillate seeds in each cell of the big indehiscent yellowish-green berry fruit, which grows to a length of 5 to 8 cm [6]. It is also usual to employ star fruits in the making of juice, pickled vegetables and salads [3]. Star fruit has been widely utilized in the treatment of a variety of disorders, including inflammation,

hypotension, hepatotoxicity, and tumours, among others [7]. The current research is focused on the preliminary phytochemical analysis of the seeds. It was decided to carry out HPLC on powdered crude medication in order to determine the most important qualitative and quantitative properties. It was decided to carry out HPLC on powdered crude medication in order to determine the most important qualitative and quantitative properties. The current study of *Averrhoa carambola* L. is being undertaken in order to produce a quality profile of the seeds, which will aid in the identification of crude drugs as well as the standardisation of the plant.

BOTANICAL DESCRIPTION OF *AVERRHOA CARAMBOLA* L.- [4]

Fruit type:	Tropical
Edible part:	Fruit
Shape of fruit:	Oval with 5 groves
Fruits per tree(annual):	200 pound
Texture:	Crisp
Taste:	Sweet

CLASSIFICATION- [8]

Scientific Name:	*Averrhoa carambola*
Kingdom:	Plantae
Subkingdom:	Tracheobionta (Vascular plants)
Super division:	Spermatophyta
Division:	Magnoliophyta (Flowering plants)
Class:	Magnoliopsida (Dicotyledons)
Subclass:	Rosidae
Order:	Geraniales
Family:	Oxalidaceae (Wood-Sorrel family)
Genus:	Averrhoa Adans (averrhoa)
Species:	*Averrhoa carambola* L.

VERNACULAR NAMES- [8]

Sanskrit:	Karmaranga
English:	Starfruit, Chinese gooseberry
Hindi:	Kamrakh,Karmal
Bengali:	Kamranga
Assamese:	Kordoi/ rohdoi
Gujarati:	Kamrakh
Marathi:	Karambal
Telugu:	Ambanamkaya
Tamil:	Thambaratham/Tamarattai
Malayalam:	Caturappuli

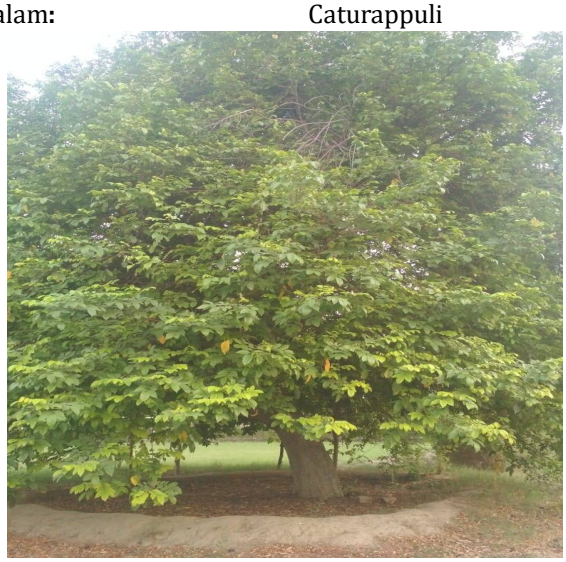

Figure 1: *Averrhoa carambola* L. Tree Figure 2: Leaves of *Averrhoa carambola* L.

Figure 3: Seeds of *Averrhoa carambola* L.

Figure 4: Fruit of *Averrhoa carambola* L.

MATERIAL AND METHOD-

Collection of Plant material-

The seeds of *Averrhoa carambola* L. were isolated from their fruit capsules which was collected from local area of Raebareli, Uttar Pradesh in September and were authenticated from Department of Pharmacognosy & Phytochemistry, Integral University, Lucknow. The voucher specimens were recorded for future use.

Extraction of plant material

Researchers use the seeds after drying them in the shade. *Averrhoa carambola* l. Seeds were coarsely crushed and macerated for 72 hours with petroleum ether, ethyl acetate, ethanol, and water separately. The spent solvent was replaced with fresh solvent every 24 hours. During the extraction process, there was some shaking. Using a rotary evaporator, the obtained extract of seeds *Averrhoa carambola* l. Were filtered, pooled, and concentrated under vacuum.

Chemicals and reagents—All of the chemicals and reagents that were used, were of the highest quality available.

PHYTOCHEMICAL SCREENING-

Preliminary phytochemical studies are carried out to detect various elements contained in Petroleum ether, Ethanolic, Ethyl acetate, and Aqueous extracts extract, such as alkaloids, glycosides, flavonoid, carbohydrates, and others, for the different pharmacological effects they have. Standard methods were used to conduct chemical tests. [9,10]

1. Alkaloids Test

a. Mayer' s test

One or two drops of Mayer's reagent are added to a couple of millilitres of plant extract. and the mixture is brushed down the walls of the test tube. When alkaloids are present, white, creamy precipitate is seen on the surface of the water

b. Wagner's test

The plant extract is diluted with Wagner's reagent. before it is placed into the test tube. Precipitation that appears to be dark crimson indicates that the test was successful.

2. Test for amino acids

An amino acid analysis is carried out by diluting the extract (10 mg) in water (10 mL), filtering using Whatmann No. 1 filter paper, & analysing the filtrate.

a. The ninhydrin assay

If the aqueous filtrate is less than two millilitres, you must add 2 millilitres of ninhydrin solution (200 millilitres acetone, 10 mg ninhydrin). The presence of a purple hue indicates a high level of amino acid content.

3. Perform a carbohydrate Test

a. Molish's test

To make a final volume of 2 mL, 2 mL of plant sample extract is mixed with 2 mL of an alcoholic solution of -naphthol. Gently add a few drops of powerful sulfuric acid to the test tube's walls while shaking, and the mixture is completely shaken. When a violet ring appears around an object, carbohydrates are present.

b. Benedict' s test

Extracts from the filter are added to the solution and incubated for 30 minutes at room temperature. Boil the mixture for two minutes in a large pot of water. The existence of sugar is indicated by a distinctive precipitate with a distinctive colour.

4. Test for Glycosides

The hydrolysate of 50 mg of extract is subjected to the following tests after it has been hydrolyzed for two hours in a water bath with concentrated hydrochloric acid.

a. Borntrager's test

Ten percent ammonia solution was added to 3 millilitres of hydrolysate filtered with chloroform. After shaking, the chloroform layer is added to the 2 millilitres of filtered hydrolysate. Glycosides are indicated by the colour of the solution.

b. Legal's test

After dissolving 50 mg of extract in pyridine, 10 percent NaOH is used to alkalize the sodium nitroprusside solution. The pink colour of glycoside is an indication of its presence.

5. Test for Phenolic compounds and Tannins

a. Ferric Chloride test

Distilled water (5 ml) is used to dilute the extract (50 mg). Adding a few drops of a 5 percent neutral ferric chloride solution completes the solution. A dark green hue indicates a high concentration of phenolic compounds.

b. Gelatin test

After dissolving the extract (50 mg) in the water and adding the Gelatin solution with 10% sodium NaCl, the mixture is ready to be used. Phenolic chemicals are indicated by the presence of white precipitate in the sample.

c. Lead acetate test

It is diluted with distilled water (50 mg) and 3 ml of 10% solution is added to the mixture. Phenolic chemicals are indicated by the presence of a large white precipitate.

d. Alkaline reagent test

The extract is processed with a 10% ammonium hydroxide solution in an aqueous solution. Flavonoids are visible as yellow fluorescence.

e. Magnesium and Hydrochloric acid reduction

Concentrated hydrochloric acid (dropwise) and magnesium ribbon fragments are added to the extract (50 mg) in 5 ml of alcohol. It is assumed that flavonol glucosides have been present in the presence of any reddish hue.

6.Test for Flavonoids-

a. Shinoda test: The powdered extracts were dissolved in ethanol (5 mL) and several drops of concentrated hydrochloric acid (HCL) was added to produce a solution similar to that of the Shinoda test. The magnesium turnings were then added to the solution, and the appearance of pink colour was observed to determine if it was a result of the process.

b. Test with lead acetate solution: A tiny amount of the aforesaid residue was mixed with a solution of lead acetate, and the resulting precipitates were inspected for the appearance of yellow-coloured particles.

7. Test for phytosterols

a. Libermann-Burchard's test

Acetic anhydride solution should be used to dilute 50 mg of the extract (an alcohol). Concentrated sulphuric acid is gently introduced to the test tube in this manner. Phytosterols can be seen in the form of a wide range of colour changes.

8. Test for Proteins

In order to determine if the extract contains any proteins, a protein assay is performed on the diluted extract (100 mg), which is filtered through Whatmann No. 1 filter paper.

a.Millon's test A few drops of Millon's reagent are added to 2 ml of filtrate to make Millon's reaction. The existence of proteins is indicated by a white precipitate.

b.Biuret test: Two millilitres of filtered filtrate are mixed with a 2 percent copper sulphate solution. Extra potassium hydroxide pellets are then added, followed by the addition of 1 mL (95% ethanol). If you see a pink colour in the ethanolic layer, then you know that protein is evident.

9. Test for saponins

Diluting the extract (50 mg) with water to a volume of 20 mL, it can be prepared. Using a graduated cylinder to shake the suspension for 15 minutes is advised. Saponins are distinguished by a two-centimeter-thick layer of foam.

Table 1: Preliminary phytochemical screening of different extract of seeds of *Averrhoa carambola* L

S.N.	Chemical Test	Petroleum Ether	Ethyl Acetate Extract	Ethanolic Extract	Water Extract
1	**Test for Alkaloid**				
	a) Mayer's reagent	+	+	+	+
	b) Wagner reagent	+	+	+	+
2	**Test for Amino Acids**				
	a) Ninhydrin test	-	+	+	-
3	**Test for Carbohydrates**				
	a) Molisch's test	+	+	+	+
	b) Fehling's Test	-	+	+	+
4	**Test for Glycosides**				
	a) Borntrager's test	+	+	+	+
	b) Legal test	+	+	+	+
5	**Test for Phenolic compounds and Tannins**				
	a) Ferric Chloride test	-	+	+	+
	b) Gelatin test	+	+	+	+
	c) Lead acetate test	+	+	+	+
	d) Alkaline reagent test	+	+	+	+
	c)Magnesium &Hydrochloric acid reduction	+	+	+	+
6	**Test for Flavonoids**				
	a) Shinoda Test	-	+	+	+
	b) Lead Acetate test	+	+	+	+
7	**Test for phytosterols**				
	a) Libermann-Burchard's test	-	+	+	-
8	**Test for Proteins**				
	a) Millon's test	-	+	+	+
	b) Biuret test	+	+	+	+
9	**Test for Saponins**	-	-	-	-

Note:(+) means present and (–) means absent)

Determination of Total Phenolic and Flavonoids Content & Antioxidant Activity-

Total Phenolic Content- [11]

The total phenolic content of an extract was determined using the Folin–Ciocalteu method. Crude extracts of 1 mg/mL were mixed for 3 minutes with Folin–Ciocalteu solution and 20 percent (w/v) sodium carbonate, and the mixture was centrifuged for 30 seconds. This was followed by another 3 minutes of mixing. It was tested for absorbance at 650 nm after an additional 60 minutes in the dark to ascertain its absorbance value. A gallic acid equivalence per gramme of dry weight is the most common unit of measurement for total phenolic content, with milligrammes per gramme of gallic acid equivalent per gramme of dry weight being the second most common.

Total Flavonoid Content- [11]

Flavonoid concentration was determined using an aluminium chloride colorimetric technique. As a final step, the mixture of 50 mL of crude extract (1 mg/mL of Ethanol) was mixed with Methanol, 4mL of distilled water, and 0.3 percent $NaNO_2$ solution; the mixture was allowed to stand for 5 minutes before being added 0.3 percent $AlCl_3$. Table 1 shows the results. It was necessary to raise the volume of the mixture to 10 mL after adding 2 mL of a NaOH solution containing 1 mol/L NaOH. 510 nm absorbance was found to be achievable after 15 minutes of resting time. The sample's flavonoid content was quantified in terms of total flavonoids.

Table 2: Total phenolics and flavonoids content of ethanolic extract of *Averrhoa carambola* L.

S.N.	Test	Mg/g
1	Total Phenolic Content	12.03
2	Total Flavonoid Content	9.75

Antioxidant Properties- [11]

An assay for determining the extract's antioxidant activity, known as the DPPH assay, was used. In comparison to the standard DPPH assay, a few minor changes were made to this one. Experiments were conducted using 200 litres of each extract (ranging from 100 to 500 grammes per litre) that were mixed with an equal volume of the DPPH solution and kept at room temperature for 60 minutes. At a wavelength of 517 nm, the absorbance of the mixture was then measured for the second time. A positive control, ascorbic acid, was used to ensure the accuracy of the results. To find out if the sample could neutralise the DPPH radical, the following tests were carried out:

$$\text{DPPH scavenging effect} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Table 3: DPPH content of ethanolic extract of *Averrhoa carambola* L.

S.N.	Test	%
1	Anti-Oxidant Activity (DPPH)	40.92

High Performance Liquid Chromatography (HPLC) of *Averrhoa carambola L. Extract:*

This was done by dissolving 10mg of powdered plant extract from *Averrhoa carambola* in 10ml of alcohol and Ethyl-Acetate to get a final concentration of 1mg/ml, then filtering the solution through a millipore 0.45-micron syringe filter (millipore). HPLC confirmed the presence of quercetin in both the ethanolic and ethyl acetate extracts. Samples were analysed by HPLC (Water) using a C18 column with dimensions of 4.6 150 mm, a flow rate of 1.0 mL/min, an injection volume of 20 L, and a detector of 273 nm. Aceto nitrile, water, and acetic acid made up the isocratic system's mobile phase (10:90:0.2, v/v).

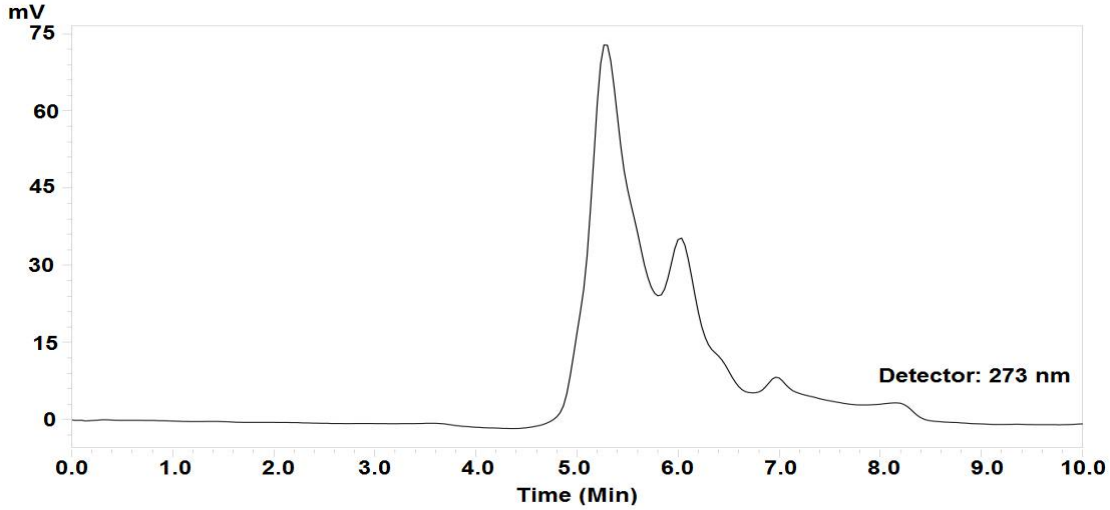

Fig No 1. HPLC chromatogram of Ethanolic extract

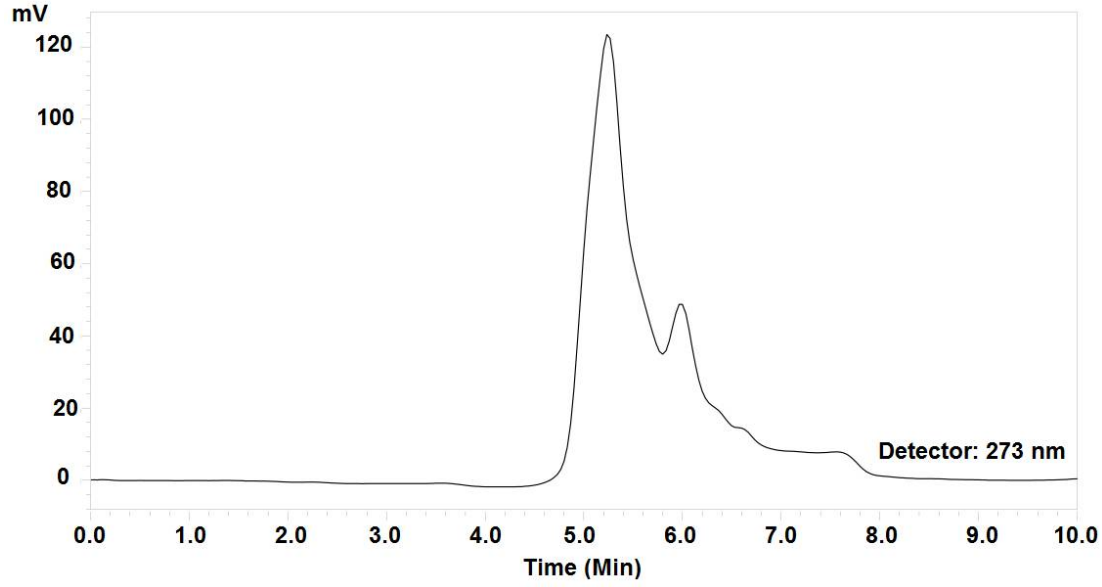

Fig. No 2 HPLC chromatogram of Ethyl acetate extract

RESULTS AND DISCUSSION

The standardization and classification of herbal pharmaceuticals is a topic that has piqued the scientific attention of the herbal drug business on a consistent basis over time. It is a critical indicator of the quality, purity, and authenticity of a product [1-2]. The macroscopical inspection of *Averrhoa carambola* L. seed was carried out as part of a standardised study as part of the standardisation study. As there is no pharmacognostic research on this medicinally effective plant seed that has been documented, the present work was done in order to develop the standards that could be useful in determining the validity of the seed. Analysis of seed extracts from *Averrhoa carambola* L. shows the presence of a wide range of phytochemicals in each of them [3-4]. The alkaloids, tannins and flavonoids were found to be present in petroleum ether, ethanolic, ethyl acetate and aqueous extracts. It was determined that the *Averrhoa carambola* L. had DPPH antioxidant potential by conducting a DPPH test, seeds are used in this study. Free radicals such as DPPH do not dimerize like other free radicals because the molecule's free electron is dissociated [5-6]. The Folin–Ciocalteu method was used to measure the ethanolic extract's total phenolic content. Antioxidants can be found in phenolic compounds because of their redox properties. There are many ways to quickly screen for antioxidant potential, and the total phenolic content can be used as an indicator. The DPPH antioxidant capacity assay was used in this study to evaluate the antioxidant activity of crude extracts from *Averrhoa carambola* L. DPPH is a stable free radical, as shown by the Folin-Ciocalteu method, due to the delocalization of the spare electron on the molecule. Antioxidant properties can be found in secondary metabolites, such as phenolics and flavonoids, because of their redox capabilities and chemical structures [7-8]. The *Averrhoa carambola* L. ethanolic seed extract proven a high antioxidant activity in the face of so many free radicals. Because of its simplicity, the DPPH radical is frequently used to assess the activity of free radical scavengers. Bioactivity is enhanced by the high phenolic and flavonoid content of these extracts. Flavonoids scavenge free radicals such as singlet oxygen and other antioxidant molecules. It has become increasingly important to create and develop standardisation procedures that are easy to use and inexpensive to implement as new chromatographic technologies are introduced to the market [9-10]. In order to standardise the ethanolic extract of *Averrhoa carambola* L., a sensitive and accurate tool known as high performance liquid chromatography (HPLC) is used. When it comes to testing raw extracts and their resulting products, HPLC is a highly effective method. The seed of *Averrhoa carambola* L. was found to be an excellent source of flavonoid after quantitative testing using HPLC [11].

CONCLUSION

A very essential laboratory or scientific process is phytochemical analysis. This technique detects vital plant parts such as seeds, bark, leaves, stems, and roots. In spite of the fact that we don't have any idea how many medicinal plants and herbs are used elsewhere in the world, they are essential to both traditional and modern medicine. Following the findings of this study, this can be stated that the standardisation and qualitative phytochemical investigation of *Averrhoa carambola* L. seed produced a set of standards that can be used to determine the identity, authenticity, and quality and purity of plant material in future researches. It would also aid in differentiating *Averrhoa carambola* L. seeds plant material from that of similar species. This research is significant, and it will inevitably require a long-term study to assess the seed's medicinal efficacy and toxicity in order to establish it as a medication. Selection and quantification of active principles will be emphasised in future research. These investigations can also assist the Ayurveda maker in determining the quality, purity, and safety of the materials used in diverse formulations.

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