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A Qualitative Analysis of *Averrhoa carambola* Leaves by GC-MS Analysis

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ABSTRACT

Natural remedies were employed long before the development of modern medicines. For thousands of years, humans have relied on medicines made from plants and animals to combat illness and infection. A phytochemical is the component that drives all of this activity and it is the one accountable for all of this. The phytochemicals in *Averrhoa carambola* are the primary focus of this study. During the phytochemical analysis, it is found that *Averrhoa carambola* leaves consist of different kinds of alkaloids, flavonoids, steroid, tannins and caroternoid. The best solvent for plant extraction was hot water, followed by cold water, even though flavonoids could not be extracted from the leaf extracts. Although there was no statistically significant difference between the amounts of alkaloids in extracts made from washed and unwashed leaves, the former contained somewhat more alkaloids (7.32% in hot water extracts and 6.83% in cold water extracts from washed leaves) than the latter (6.12% in hot water extracts and 5.32% in cold water extracts from unwashed leaves). The flavonoids in bitter leaf were released by both ethanolic and hot water extracts. Both extracts were used to identify carotenoid pigments, but the amounts were so similar as to be negligible. The research confirmed that bitter leaf contains antibacterial activity.

Keyword: *Averrhoa carambola* leaves, Alkaloids, Flavonoids, Steroid, Tannins, Caroternoid.

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Introduction

As per recent reports, several people have recently returned to conventional medicine, including natural products, because of their efficacy in preventing and curing illnesses with few to no side effects [1]. Plant *Averrhoa carambola* belongs to the family recognized as being native to tropical Indian subcontinent and Southeast Asia and cultivated throughout the tropics for its edible fruit [2]. The *Averrhoa carambola* L. tree bears fruit that can be consumed and thrives in a variety of tropical and subtropical climates. It's a contentious fruit because of the high levels of oxalate it contains, despite its nutritional worth and purported health benefits [3,4]. The plant is of great scientific interest since it has been hypothesized to have powerful phytochemicals with potential medical applications. *Averrhoa carambola* is a shrub or small tree usually branched near the base, 2-10 m high, bark rough with dense black streaks and grows under a range of ecological zones [2]. The *Averrhoa carambola* fruit has been used for many different purposes in traditional medicine, including as an anti-parasitic agent, an appetizer and a digestive tonic [5,6]. According to traditional Indian medicines, the ripe fruits of the *A. carambola* tree are a good therapy for hemorrhoids, as well as for eczema, fever, and diarrhea [7]. In addition, Ayurvedic practitioners typically use the mature fruit of A. carambola for its digestive and tonic properties [8]. The roots, fruits, and leaves of *A. carambola* have a long history of use in Traditional Chinese Medicine (TCM) for the treatment of a wide variety of conditions and are increasingly recognized as an effective herbal medicine for stimulating kidney function and reinforcing the masculine, activeness and fresh mind [9].

Experimental

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Triple beam balance of 2500 g capacity, beakers, Whatman filter paper No. 1 (125 mm diameter), funnel, spatula, conical flask, mechanical grinder, water ignition tube, thin-layer chromatography plate, capillary tube, Thin-layer chromatography tank, refrigerator, cork Gas Chromatograph –Mass Spectrometer were used in this experimental work. The chemicals were procured from the various reputed commercial sources and of Analytical grade.

Instruments and Conditions

The GC-MS analysis of the extract was carried out using a HP7890GC instrument integrated with an Agilent 5975C MSD mass spectrometer (Agilent Technologies, Santa Clara CA, USA). The capillary column was an Agilent HP-5MS (30 m × 0.25 mm i.d. × 0.25 Nm film thickness), helium (purity > 99.999%) was used as the carrier gas, and the flow rate was 1 mL min^{-1}. The injector temperature was 250 ºC and the injection mode was splitless. The GC oven temperature was held at 50 ºC for 5 min, which was increased 210 ºC at a rate of 3 ºC/min and finally increased to 230 ºC at @15 ºC/min. The mass spectrometer conditions were (12, 13, 14) ionization energy, 70 Ev; ion source temperature, 230 ºC; the quadrupole temperature, 150ºC; quadrupole mass spectrometer scan range 30-500 atomic mass unit (amu); solvent delay time 2.8 min.

Collection of Samples

Fresh leaves of *Averrhoa carambola* (Fig. 1) were collected from the local garden of Faizabad city and authenticated at the herbarium of Department of Botany of R.M.L Avadh University.

Before being ground into powder in an electric blender, the seeds/leaves were often removed by hand from the fruit pulp, rinsed and dried at room temperature. The *A. carambola* leaves were taken off the stem after harvest, rinsed in distilled water to get rid of any remaining dust and debris, and then dried at room temperature. Mechanically ground

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dried leaves were next weighed and totally submerged in methanol (maceration or cold extraction method) for 72 h. Using Whatman filter paper no. 1, the mixture was separated into its liquid and discarded the solid components. The concentrated filtrate was then heated to 40 ºC in a water bath. The % yield was determined by weighing the dried sample and plugging the numbers into the following formula:

$$\text{Yield (\%)} \frac{weight\ of\ extract\ yield}{weight\ of\ dry\ sample}\ x\ 100$$

Fig. 1 Fresh and dried leaves of *Averrhoa carambola*

Phytochemical screening (qualitative analysis)

The crude aqueous extracts of *Averrhoa carambola* leave were tested for the presence of alkaloid, flavonoid, steroids, carotenoids and saponin.

Test for alkaloid (Wanger Test): Wagner Dragenderoffs Brown precipitate which turns intense yellow with the picric acid whereas in case of tannins, upon the addition of ferric chloride, the colour changes from greenish to black precipitate.

Test for Flavonoids: This was determined according to the standard method, in brief 300 g of dried leaves was boiled in 150 mL of 2 M HCl solution for 30 min under reflux. It was

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allowed to cool and then filtered through Whatman No 42 filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with a drop. The flavonoids precipitated were recovered by filtration using weighed filter paper. To a volume of mL of extract, two to three drops of sodium hydroxide were added. After the addition of a few drops of dilute HCl, the substance, which had initially had a deep yellow color, eventually became colorless, which served as an indicate the presence of flavonoids.

Test for Saponin/Frothing test: Shaked the mixture of 100 mL containing 5 g of plant extract well until a steady foam forms. The existence of saponin was inferred from the fact that the foaming continued even after the water was heated.

Test for Tannin: After treating approximately 4 g of plant extract with 100 mL distilled water, the resulting filtrate was treated with ferric chloride reagent. Tannin can be identified by the presence of a dark blue-black precipitate.

Test for carotenoids

Using a simple mixer, a predetermined quantity of each sample was mixed together with ethanol until it was uniform in consistency. The mixture that was employed was a 1:10 ratio. The initial crude extracts were obtained by filtering the homogenate in order to get them. After thoroughly mixing the filtrate, 25 mL of ether were added to it, and this was followed by the application of 25 mL of distilled water in a separating funnel. This was done so that the carotenoids could be separated by the ether. The other layer was salvaged, then put into a vacuum desiccator where it was heated to a low temperature (between 35 and 50 ºC) and allowed to evaporate until it was completely dry. After that, the dry extract was saponified with 30 mL of ethanolic KOH, and it was put away in a dark closet for the night. The carotenoids were dissolved in 25 mL of ether the following day, and after that, they were

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rinsed in two equal parts of 25 mL of distilled water. After drying the carotenoid extract in a desiccator to remove the ether layer, it was subsequently treated with a light petroleum and left to stand at -10 ºC overnight. The following day, the precipitate steroid was separated out using centrifugation and the carotenoid extracts were evaporated to dryness.

Thin-Layer Chromatography (TLC)

The methanolic extract of *Averrhoa carambola* was subjected to thin layer chromatography on a silica gel-coated glass TLC plate. The TLC plate was trimmed to 10 cm × 5 cm, and the starting point was marked with a pencil scribble about 1 cm up from the plate's bottom border. The dried extract was reconstituted with methanol. In the development chamber, the solvent system of hexane, chloroform, and ethyl acetate was prepared. The chemicals present in the extract separate out and appear as spots on the plate as the solvent, which is the eluent, flows through the plate and rises due to the capillary action of the plate. Compounds further from the origin show more motion and are less polar, while those nearer the origin show less movement and are more polar. Then, daylight and iodine crystal vapour in a dark room were used to observe the dots. Different solvent volumes and ratios were tried until a high-quality separation was achieved. The following equation was used to obtain the retention factor (R_f).

$$Rf = \frac{distance\ moved\ by\ the\ solute\ (i.e.\ the\ pigment}{distance\ moved\ by\ solvent\ (known\ as\ solvent\ front)}$$

Results and Discussion

The *Averrhoa carambola* plant is known to have common phytochemical components, which are non-nutritive plants and are widely employed in traditional medicine for the treatment of a variety of conditions. According to a scientific classification, the plant is considered to be a

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member of the Kingdom Plantae. The leaves are dark green in color, and they smell and taste characteristically bitter. As a result, the purpose of the research is to determine how much of a particular class of phytochemical compounds are found in the extracts of *Averrhoa carambola* bitter leaves.

The methanolic leaves extract of *Averrhoa carambola* displayed the substantial positive phytochemical results, as can be seen in Table-1. These results were evidenced by remarkable colour changes. In the majority of tests that were examined, the flavonoids, alkaloids, and phenols were found to be in the high concentrations.

Table-1 Phytochemical screening results of methanolic leaves extract of *Averrhoa carambola*

Parameter	**Observation**	**Methanol extract**
Alkaloid	Formation of reddish-precipitate indicated the presence	+
Tannin	No colour change was observed and forth was observed	+
Flavonoid	A red colour indicates the presence of flavonoid	+
Saponin	Formation of 1cm layer of foam was observed	+
Terpenoid	A brown ring was observed at the junction	+

Thin layer chromatography: On examination TLC with silica gel GF_{254} plate and mobile phase *viz.* toluene:ethyl acetate:methanol:glacial acetic acid (7.5:1.5:0.8:0.2), the results are shown in Fig. 2 and Table-2.

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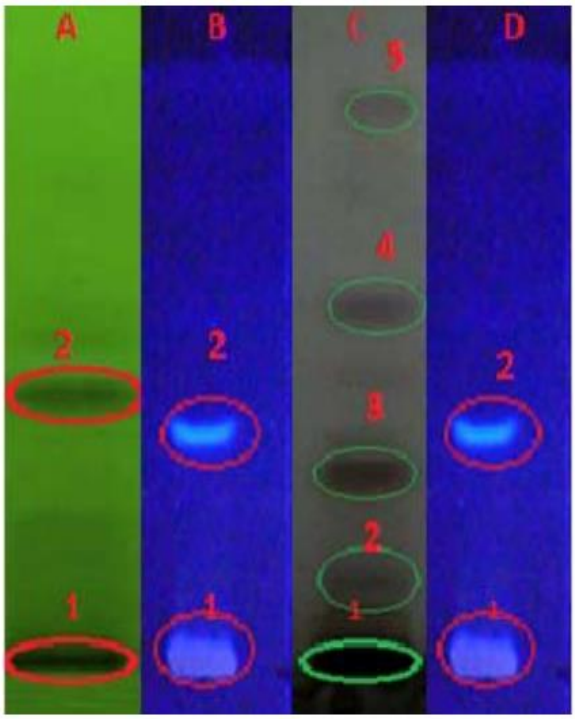

Fig. 2: TLC profile of methanolic leaves extract of *Averrhoa carambola*

Table-2 Result from thin layer chromatography

Solvent system used	Distance moved by solvent (cm)	Distance moved by pigment (cm)	Retention factor value (R_f)
Methanol:chloroform: Ethyl acetate (5:2:3)	7.6	6.6	0.80
		5.7	0.75
		4.4	0.58
		1.9	0.22
		1.0	0.13
		0.6	0.08

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GC-MS analysis: Eight bioactive phytocomponents were found in the *Averrhoa carambola* leaf extract after GC-MS analysis (Fig. 3), with bis(2-ethyhexyl)phthalate having the highest quantitative content (24.61%). Triethyl citrate, on the other hand, was found to have the lowest compositional percentage (0.59%) and retention time of 17.758.

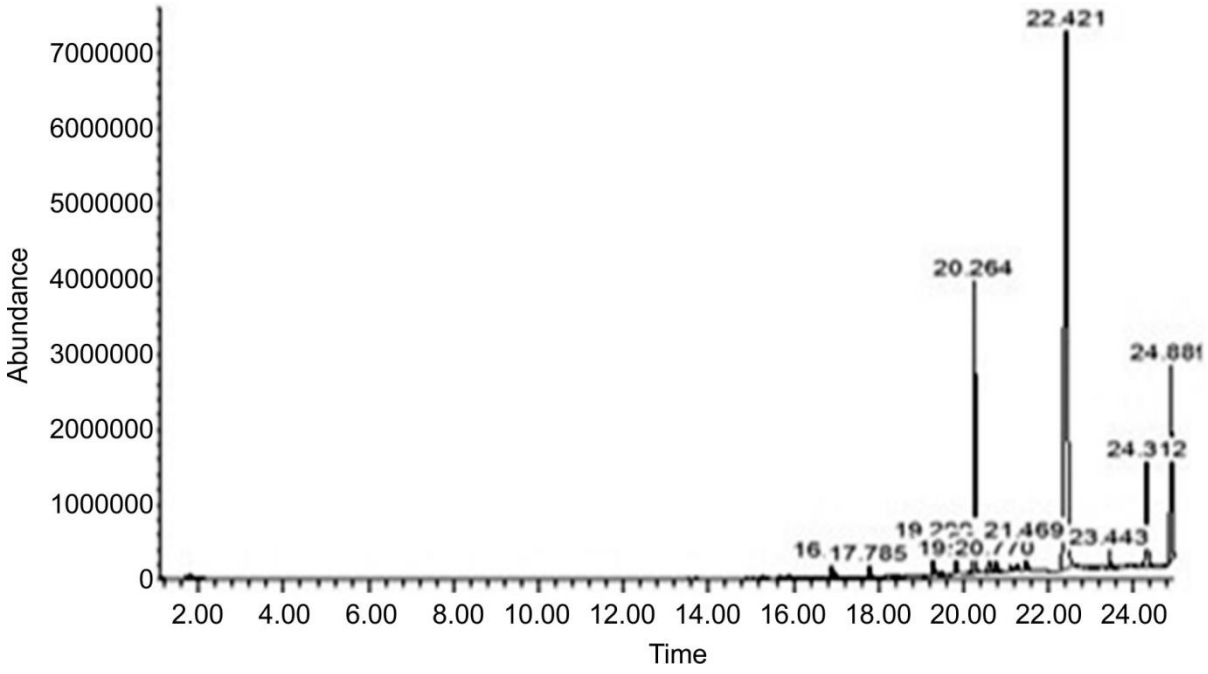

Fig. 3 Gas chromatographic profile of Averrhoa carambola methanolic leaves extract

Table 1 Bioactive Phytocompotents Identified in GC-MS Analysis of *Averrhoa carambola* Leaf Extract

S.No.	Compound	Molecular Formula	Total area %
1	1-Hexadecene	$C_{16}H_{32}$	0.67
2	Triethyl citrate	$C_{12}H_{20}O_7$	0.59
3	1-Octadecene	$C_{18}H_{36}$	1.35
4	Dibutyl phthalate	C_6H_6O	14.57
5	Behenic alcohol	$C_{22}H_{46}O$	1.08
6	Bis 2-ethylhexyl phthalate	$C_{24}H_{38}O_2$	24.61
7	1-Docosene	$C_{22}H_{44}$	10.31

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Natural remedies were employed long before the advent of modern medicine. For thousands of years, humans have relied on antibacterial and curative substances derived from plants and animals. The phytochemical in it is the magic ingredient. The purpose of this research was to learn more about the phytochemical profile of *Averrhoa carambola*. Alkaloids, flavonoids, steroids, tannins, and caroterpenoids were found in the leaves of *Averrhoa carambola* during a phytochemical analysis. Although they were unsuccessful in extracting flavonoids from bitter leaf extracts, methanol proved to be the most effective solvent of extraction for the plants. There were somewhat more alkaloids in the extracts made from cleaned bitter leaves compared to those made from unwashed leaves.

CONCLUSION

The results from this work has shown that the methanolic extracts of the plants under study contains many phytochemical compounds which include alkaloids, steroids, flavonoids, carotenoids and tannins which are responsible for their various activities and also may account for medicinal benefits. It can be concluded from this work that the different solvents of extraction have varying abilities to liberate these compounds and the quantities that each liberate has been ascertained. The selected plants contain substantial amount of phytochemical which are helpful in the prevention of some deadly diseases. *Averrhoa carambola* leaves could help fulfill the growing demands of plant based foods for human nutrition.

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