Research Article

Phytochemical Screening, TLC Profiling and GC-MS Analysis of the Methanolic Extract of *Averrhoa carambola* Leaves

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ABSTRACT

***Background and objectives**: Averrhoa carambola* is a member of the Oxalidaceae family and is grown extensively throughout Southeast Asia. It has important uses in ethnomedicine and ethnobotany. The present study was designed to determine the bioactive compounds in the methanol leaf extract of *Averrhoa carambola*.

Methods: Various bioactive components were found in the *Averrhoa carambola* leaf extract during phytochemical screening. Thin layer chromatography (TLC) was carried out in silica gel plates using a variety of mobile phase techniques, toluene, ethyl acetate, chloroform and methanol and to detect with different colored phytochemical compounds. A Shimadzu GC-MS/MS system (Model: GCMS-TQ8040) was used to analyze the methanol extract of *Averrhoa carambola* leaves using gas chromatography-mass spectrometry (GC-MS).

Results: In the present investigation, qualitative phytochemical screening of methanol leaf extract was confirmed the presence of many phytochemicals like alkaloids, flavonoids, triterpenes, anthraquinones, anthocyanins, reducing sugar, phenols and so on. Thin Layer chromatography (TLC) of the methanol leaf extract was performed for six important phytochemicals alkaloids, tannins, flavonoids, triterpenes, coumarin and anthraquinones. The chromatographic analysis of the extract revealed 83 ingredients, of which 40 compounds were newly identified from the plant extract. Thioglycolic acid, S-isopropyloxycarbonyl constituted the predominant component at 11.28%, whereas Triacontane, 1-iodo exhibited the minimal peak area at 0.09%.

Conclusion: The results of this investigation provide the way for the advancement of herbal medicines using *A. carambola*, potentially leading to the discovery of novel drugs for various ailments.

Keywords: *Averrhoa carambola*, Phytochemical, Thin Layer Chromatography, GC-MS.

INTRODUCTION

Averrhoa carambola, commonly known as starfruit for its distinctive cross-sectional appearance is a tropical fruit-bearing tree in the Oxalidaceae family. Indigenous to regions such as Ceylon and the Moluccas, *A. carambola* is also native to Southeast Asia, particularly Malaysia, the Philippines, and India. Today, it is widely cultivated in tropical and subtropical regions around the world, including parts of the Caribbean, Central America, Brazil, Australia, and the United States.[1] The fruiting body of *A. carambola* is a fleshy berry with a thin, smooth, waxy outer integument that varies in color from pale green to bright yellow when ripe. Each fruit typically has five longitudinal ridges, creating a star shape when sliced crosswise, which provides the fruit with its common name.[2]

The starfruit is considered valuable in many tribes for its use in traditional medicine as well as for its unique appearance and flavor, which can range from sweet to tart. Starfruit contains quite several bioactive compounds making it to be used for medicinal purposes as well as used traditionally. Major bioactive classes include flavonoids like quercetin and kaempferol, and phenolic acids like chlorogenic and gallic acids that have anti-inflammatory, antioxidant and antimicrobial properties.[3] Tannins included in the plant exhibit astringent effects while saponins are acknowledged for their cholesterol-reducing and immune-enhancing capacity, alkaloids on the other hand can relieve pain and help with diabetes.[4]

Fruit contains epicatechin, proanthocyanidins, organic acids, sugar and polyols.[5,6] Along with vital elements like potassium and magnesium that support heart and bone health, the fruit is also a substantial source of vitamin C, which supports immunological function and skin health. The fruit's potential antibacterial and anti-inflammatory qualities are highlighted by the fact that it is frequently used to treat ailments like malaria.[7,8]

Additionally, the root is used for curing chronic headache, arthralgia and the leaves for boils, gastroenteritis, and traumatic injury. Previous studies showed that *Averrhoa carambola* exhibits important pharmacological activities including anti-oxidant, anti-inflammatory, anti-microbial, analgesic, anti-diabetic and anti-diarrheal properties.[9-14] The abundance of bioactive compounds makes this plant ideal for various pharmacological and phytochemical studies. The present work aims to investigate the phytochemical content of *A. carambola* grown in Bangladeshi tropical weather through TLC and GC-MS analysis.

MATERIALS AND METHODS

Collection of plant material

The fresh leaves of *Averrhoa carambola* were collected on 23 July 2023 during the daytime from the Bangladesh Bank Officers Quarters, Banani, Dhaka. The plant was identified and authenticated by a senior scientific officer at the Bangladesh National Herbarium, Mirpur-Dhaka. The accession code assigned to the plant is DACB 94779. During the collection, care was taken to separate the desired plant parts from any undesirable materials or other plant parts to ensure the purity of the sample.

Plant extraction

2 kg of fresh *Averrhoa carambola* leaves were collected, and any surface impurities were properly cleaned with distilled water. The cleaned leaves were then dried under shade for a period of 7-8 days. Once dried, the leaves were ground into a fine powder using a blender machine, yielding 500 g of powdered material. The powdered leaves were soaked in 4 L of methanol in an amber glass jar, which was kept at room temperature for 14 days. During this period, the jar was manually shaken in a clockwise direction to ensure thorough mixing. After the extraction period, the mixture was filtered using Whatman No. 1 filter paper to separate the liquid extract from the solid residue. The filtered extract was then concentrated using a water bath maintained at 45°C to evaporate the solvent. The resulting concentrated extract was further dried and stored under refrigeration at 4°C for subsequent analysis.

Preliminary phytochemical screening

The methanolic extract of *Averrhoa carambola* L. leaves was subjected to a series of qualitative tests to identify the presence of various phytochemical constituents.

Test for Alkaloids:

Mayer's Test: 2 ml of the extract solution was mixed with 0.2 ml of dilute hydrochloric acid and 0.1 ml of Mayer's reagent. An abundance of alkaloids was detected by a precipitate with a yellowish buff color.[15]

Hager's Test: 2 ml of the extract solution was mixed with 0.2 ml of dilute hydrochloric acid and 0.1 ml of picric acid solution (Hager's reagent). The production of a yellowish precipitate indicated the existence of alkaloids.[16]

Dragendorff's Test: 0.1 ml of Dragendorff's reagent and 0.2 ml of diluted hydrochloric acid were mixed with 2 ml of the extract solution. When an orange-brown precipitation formed, it meant that alkaloids were detected.[17]

Test for Steroids:

1 ml of chloroform was used to dissolve 10 mg of the extract. Steroids were present because the chloroform layer became reddish-brown when 1 ml of sulfuric acid was added, and the acid layer fluoresced green.[18]

Test for Flavonoids:

10% sulfuric acid was used to hydrolyze the extract, and ether was then used to extract i. The ether extract was divided into three portions:

Ammonia Test: 1 ml of dilute ammonia solution was added, resulting in a greenish-yellow color, indicating the presence of flavonoids.

Sodium Carbonate Test: 1 ml of dilute sodium carbonate solution was added, resulting in a pale-yellow color, indicating the presence of flavonoids.

Sodium Hydroxide Test: 1 ml of dilute sodium hydroxide solution was added, resulting in a yellow color, indicating the presence of flavonoids.[19]

Test for Reducing Sugars:

Fehling's Test: 5 ml of Fehling's A and B solutions were mixed with 5 ml of the extract solution and boiled for 5 minutes. The absence of a brick-red precipitate indicated the absence of reducing sugars.

Alpha-Naphthol Test: 5 ml of the extract solution was mixed with 2 drops of 5% alpha-naphthol solution and 1 ml of sulfuric acid was added along the sides of the test tube. There were reducing sugars present because a violet-colored ring appeared where two liquids interacted.[20]

Test for Saponins:

1 ml of the extract solution was diluted to 20 ml with distilled water and shaken for 15 minutes. Saponins were detected because a 1-centimeter layer of foam formed.[21]

Test for Tannins:

5 ml of the extract solution was mixed with 1 ml of 10% potassium dichromate solution. The formation of a yellowish-brown precipitate indicated the presence of tannins.

Test for Glycosides:

1 ml of water and a small amount of the extract was mixed with a few drops of sodium hydroxide solution. The formation of a yellow color indicated the presence of glycosides.

Test for Carbohydrates:

2 ml of the extract was mixed with 2 ml of concentrated sulfuric acid. The formation of a red or reddish-violet ring indicated the presence of carbohydrates.[22,23]

Test for Anthocyanins:

HCl test: For the HCl test, 2 mL of the extract was combined with 2 mL of 2N HCl, followed by the addition of a few drops of ammonia. The appearance of a pink-red color, which changed to blue-violet upon the addition of ammonia, confirmed the presence of anthocyanins.[24,25]

Test for Anthraquinones:

Borntrager's test: For Borntrager's test, a few milliliters of the filtrate were mixed with 10 mL of 10% ammonia solution and shaken vigorously for 30 seconds. Anthraquinones were detected because a reddish-colored solution appeared.[26-28]

Test for Terpenoids:

2 mL of chloroform was mixed with 5 mL of the plant extract and evaporated using a water bath. After evaporation, 3 mL of concentrated sulfuric acid (H_2SO_4) was added, and the mixture was boiled in a water bath. The appearance of a gray-colored solution confirmed the presence of anthraquinones.[27]

Thin-layer chromatography (TLC) method

Thin Layer Chromatography (TLC) is a highly versatile separation method that is widely used for both qualitative and quantitative sample analysis. TLC can be used to analyze virtually any substance class, including steroids, alkaloids, glycosides, carbohydrates, flavonoids, terpenoids, anthraquinones, anthocyanins and so on.[29] The leaf extract was analyzed with (TLC) using silica gel 60 as the stationary phase. The TLC profiling was determined as described by Wagner and Bladt, 1996. Four solvents were used for comparison of the extraction efficiency: (1) toluene (2) 10% ethyl acetate (3) chloroform and (4) methanol. The plant extract was transferred to a pre-coated TLC plate via capillary tubes, utilizing multiple solvents of increasing polarity. A light line was drawn on the TLC plate to mark the baseline, with small dots indicating the positions for sample application. After developing the plate in the respective mobile phase, it was removed, air-dried, and examined under ultraviolet light at 254 nm and 365 nm to observe any fluorescence or absorption patterns. They were later sprayed with 1% vanillin in concentrated H_2SO_4 solution followed by heating at 105°C**.** Following plate drying and exposure to 1% vanillin in concentrated H_2SO_4, all plates were viewed using UV light, allowing for the observation of all different spots.[18]

Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis of the methanolic extract was performed using a Shimadzu GC-MS/MS system (Model: GCMS-TQ8040) equipped with a DB-5ms capillary column, measuring 30 meters in length, with an inner diameter of 0.25 mm and a film thickness of 0.25 µm. Helium was employed as the carrier gas at a flow rate of 1.0 mL/min.

Sample Preparation

For the GC-MS analysis, the methanolic extract was prepared by following specific injection parameters to ensure optimal performance. The sample underwent three rinses with pre solvent, three rinses with post-solvent, and two rinses with the sample itself. High plunger speed settings were used for both suction and injection, with a viscosity compensation time of 0.2 seconds. The syringe insertion speed was set to high, and the injection mode was set to normal, with five pumping times. The injection port dwell time was 0.3 seconds, and no terminal air gap was used. The plunger washing speed was set to high, with a washing volume of 8 µL. Both the syringe suction position and injection position were set to 0.0 mm, utilizing one solvent vial.

Instrumentation Conditions

The injection volume was set to 1 µL in splitless mode with injection temperature of 250 °C. The oven temperature program started at an initial temperature of 60°C, held for 2 minutes, then increased to 280°C at a rate of 10°C/min, and held at 280°C for 10 minutes. The ion source temperature was maintained at 230°C, and the interface temperature was set to 250°C. The solvent cut time was 3.50 minutes, with the detector gain mode set relative to the tuning rest and a detector gain of +0.50 kV. The threshold was set to 0, and data were acquired without using CID gas (Q3Scan) set to ON.

The mass spectrometric analysis was performed with the compound designated as Plant Extract, commencing at 3.50 minutes and concluding at 40.00 minutes. The acquisition was carried out in Q3 Scan mode, featuring an event duration of 0.300 seconds and a scan speed of 2000. The mass scan range extended from m/z 50.00 to m/z 600.00, utilizing the GC as the sample inlet unit. The mass spectra were recorded over the range of 50-600 m/z. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS. The total GC running time is 36.50 minutes.

Identification of compounds

The identification of compounds was performed by comparing the mass spectra with those in the National Institute of Standards and Technology (NIST) library having more than 62,000 patterns. The mass spectrum of the unknown component was compared with spectrum of known component stored in NIST library. The relative concentrations of the identified compounds were determined based on their peak areas. The name, molecular weight, retention time and peak area percentage of the test materials was ascertained. The data obtained from GC-MS analysis were processed using Shimadzu's GC-MS solution software.

RESULTS AND DISCUSSION

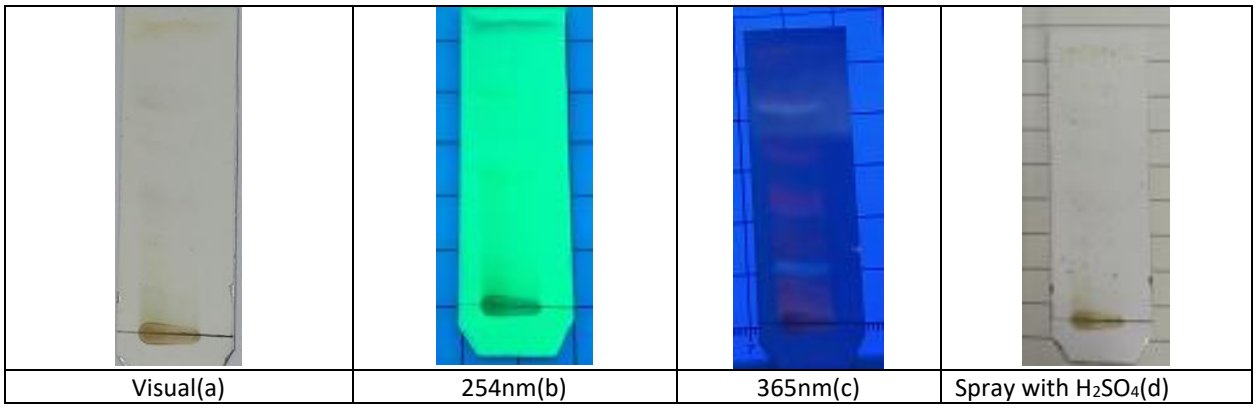
After the successful maceration process of the leaves of the plant in investigation, the preliminary phytochemical study revealed that methanolic extract of contains alkaloids, flavonoids, reducing sugars, carbohydrates, glycosides, anthocyanins, anthraquinones, terpenoids and tannins. Saponins and steroids were absent in the *A. carambola* methanolic extract, as summarized in [Table 1].

Table 1: Preliminary phytochemical evaluation of methanol extracts of *A. carambola*

Sl. no	Phytochemical constituents	Test/Reagent	Present	Absent
1	Alkaloid	Mayer's test	+	
		Hager's test		
		Dragendorf's test		
2	Flavonoid	Ammonia test	+	
		Sodium Carbonate Test		
		Sodium Hydroxide test		
3	Reducing sugar	Fehling test	+	
		Alpha-Napthol test		
4	Steroid		-	-
5	Saponin		-	-
6	Carbohydrate		+	
7	Glycoside		+	
8	Tannin		+	
9	Anthocyanin	HCl test	+	
10	Anthraquinone	Borntrager's test	+	
11	Terpenoid		+	

+ =Present; − =Absent

Figure 1: TLC screening of the methanol extracts of *A. carambola* by visual observation (a), under UV at 254 nm (b), at 365 nm (c) and finally after spraying with spray reagent (d).



Visual(a)	254nm(b)	365nm(c)	Spray with H_2SO_4(d)

Phytochemical screening of the methanolic extract of *A. carambola* revealed the presence of phyto-compounds that have been documented to have antioxidant and other activities. It has been demonstrated that flavonoids are very efficient scavengers of the majority of oxidizing molecules, such as singlet oxygen, and other free radicals that are linked to a number of illnesses.[30] Flavonoids protect mucous membranes and have anti-oxidant properties. Vegetables high in flavonoids are popular functional foods because they can be used to treat heart conditions. Because of their high bioavailability, flavonoids have been shown to provide pharmacologically significant plasma concentrations in humans when consumed consistently through diet. Furthermore, flavonoids may have cardioprotective effects against ischemia reperfusion, according to a number of studies.[31,32] Tannins lessen the mucosa's susceptibility to chemical irritation. As a result, they lessen inflammation, protect the stomach mucosa, and prevent excessive acidity. Furthermore, it has been found that alkaloids and terpenoids have strong anti-gastric ulcer properties.[33]

Typically, thin-layer chromatography is used to better identify the bioactive substances. Table 2 provides a summary of the TLC profiling results. Certain alkaloids, anthraquinones, coumarins, flavonoids, and tannins exhibit orange, red, blue, yellow, and green fluorescent zones in the chromatogram when left untreated chemically. When exposed to UV 365 nm light, coumarins exhibit blue, blue-green, and yellow-green fluorescence; anthraquinones produce a stable red color; and alkaloids appear as a stable orange color (vis.).[34] Under the same circumstances, triterpenes exhibit a bright blue color (UV 365), and tannins may produce reddish-brown zones (vis.). After being sprayed with H_2SO_4, flavonoids produce orange, red-orange, and yellow-green colors (UV 365 nm), whereas

certain coumarins and anthraquinones create yellow-green fluorescent zones.[35,36]

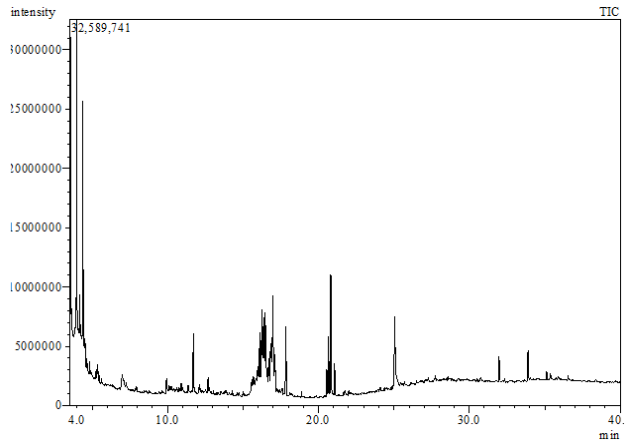
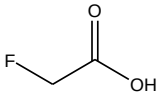
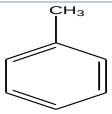
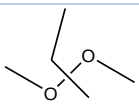
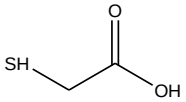
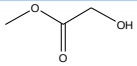
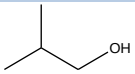
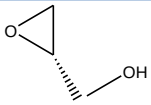
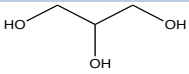
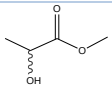
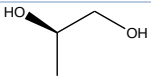
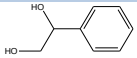
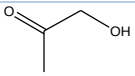
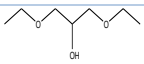
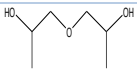
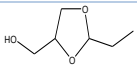
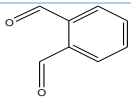
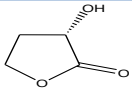
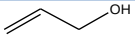
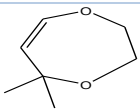


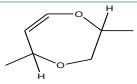
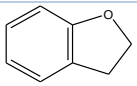
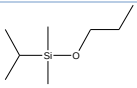
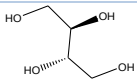
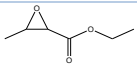
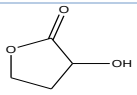
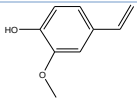
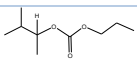
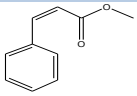
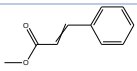
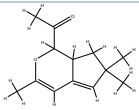
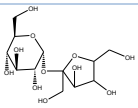
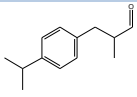
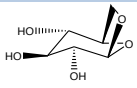
Figure 2: GC-MS chromatogram of methanolic extract of *A. carambola*

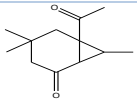
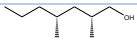
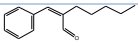
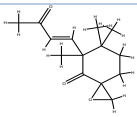
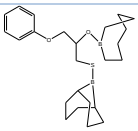
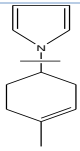
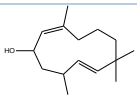
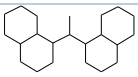
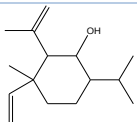
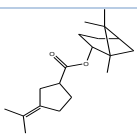
The identification of many components is the result of the GC-MS study of the methanolic extract of *A. carambola*. Mass spectrometry, together with GC, was used to identify these compounds. Table 2 showed the different components found in *A. carambola* leaves that were identified by GC-MS. Fluoroacetic acid; 2,2-Dimethoxybutane; Thioglycolic acid, S-isopropyloxycarbonyl-; Oxiranemethanol, (R)-; 1,2-Ethanediol, 1-phenyl-;2-Propanol, 1,1'-oxybis-;1,3-Dioxolane-4-methanol, 2-ethyl-;5H-1,4-Dioxepin, 2,3-dihydro-2,5-dimethyl-;2,3-Dihydroxy-3-methylpentanoic acid; 1-Dimethyl(isopropyl)silyloxypropane; Erythritol; Methyl cis-cinnamate; 1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl) ethenone; 3-(4-Isopropylphenyl)-2-methyl propionaldehyde; 6-Acetyl-4,4,7-trimethylbicyclo[4.1.0]heptan-2-one; 1-Heptanol, 2,4-dimethyl-, (R,R)-(+)-; Cinnamaldehyde, .alpha.-pentyl-;5,6,6-Trimethyl-5-(3-oxobut-1-enyl)-1-oxaspiro[2.5]octan-4-one; Propane, 2-(9-borabicyclo[3.3.1]non-9-yloxy)-3-(9-borabicyclo[3.3.1]non-9-ylthio)-1-phenoxy-;1-[1-Methyl-1-(4-methyl-cyclohex-3-enyl)-ethyl]-1H-pyrrole; 2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15-tetramethyl-, acetate, (E,E,E)-; Humulane-1,6-dien-3-ol; Naphthalene, 1,1'-ethylidenebis [decahydro-; Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-,[1R-(1.alpha.,2.alpha.,3.beta.,6.alpha.)]-; Cyclopentane carboxylic acid, 3-isopropylidene-, bornyl ester; Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-;(4aS,5S,8aS)- 5-Isopentyl-1,1,4a,6-tetramethyl-1,2,3,4,4a,5,8,8a-octahydronaphthalene; Ageratriol; 2-Buten-1-ol, 2-ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-; Neophytadiene;E)-3-Methyl-5-((1R,4aR, 8aR)-5,5,8a-trimethyl 2methylende cahydronaphthalen-1-yl)pent-2-en-1-ol; Bicyclo[4.3.0]nonane, 7-methylene-2,4,4-trimethyl-2-vinyl-;4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-; 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, [R-[R*,R*-(E)]]-; Humulenol -II;3-Isopropoxy-1,1,1,7,7,7-hexamethyl 3,5,5tris (trimethyl siloxy)tetrasiloxane; [1,1'Bicyclopropyl]-2-octanoic acid, 2'-hexyl-,methyl ester; (1S,2E,4S,5R,7E,11E)-Cembra-2,7,11-trien-4,5-diol; Cyclononasiloxane, octadecamethyl-;(R)-6-Methoxy-2,8-dimethyl-2-((4R,8R)-4,8,12-trimethyl tridecyl) chroman were present in the methanolic extracts of *A. carambola*. As shown in [Figure 1], the GC-MS spectra verified the existence of many components with varying retention periods. To determine the nature and structure of the molecules, the mass spectrometer examines the chemicals that elute at various times.

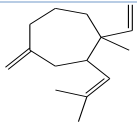
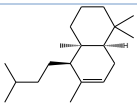
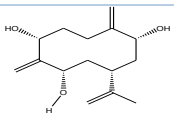
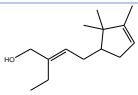
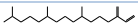
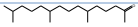
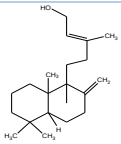
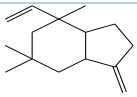
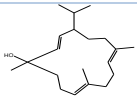
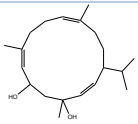
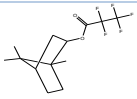
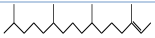
Table 2: Compounds identified in the methanolic extract of *A. carambola* in GC-MS

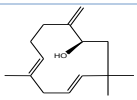
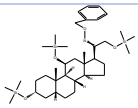
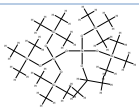
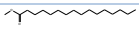
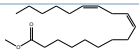
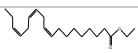
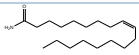
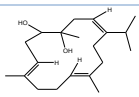
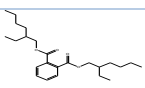
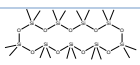
S. no	R. Time	Area%	Molecular formula	Molecular weight	Chemical structure	Compound Name	Compound Type
1	3.637	4.19	FCH_2CO_2H	78.04 g/mol		Fluoroacetic acid **(newly identified)**	Organofluorine
2	3.79	1.27	$C_6H_5CH_3$	92.14 g/mol		Toluene	Aromatic
3	3.835	3.31	$C_6H_{14}O_2$	118.174		2,2-Dimethoxybutane **(newly identified)**	Aliphatic
4	3.979	11.28	$HSCH_2CO_2H$	92.11		Thioglycolic acid, S-isopropyloxycarbonyl - **(newly identified)**	Carboxylic acid

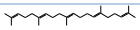
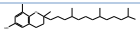
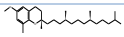
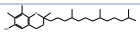
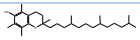
5	4.177	2.64	$C_3H_6O_3$	90.07		Acetic acid, hydroxy-, methyl ester	Glycolic acid
6	4.22	2.37	$C_4H_{10}O$	74.12		1-Propanol, 2-methyl-	Isobutyl alcohol
7	4.3	0.86	$C_3H_6O_2$	74.078		Oxiranemethanol, (R)- **(newly identified)**	Glycidol
8	4.373	6.32	$C_3H_8O_3$	92.09		Glycerin	Glycidol
9	4.434	2.51	$C_4H_8O_3$	104.10		Propanoic acid, 2-hydroxy-, methyl ester, (.+/-.)-	Carboxylic ester
10	4.496	1.04	$C_3H_8O_2$	76.09		R-(-)-1,2-propanediol	Aliphatic diol
11	4.54	0.99	$C_8H_{10}O_2$	138.16		1,2-Ethanediol, 1-phenyl- **(newly identified)**	Benzene
12	4.847	0.24	$C_3H_6O_2$	74.078		2-Propanone, 1-hydroxy-	Alpha-hydroxy ketone
13	5.256	0.21	$C_7H_{16}O_3$	148.20		1,3-Diethoxy-2-propanol	Cellulose
14	5.327	0.27	C_5H_6O	82.04		2-Cyclopenten-1-one	Alicyclic ketone
15	5.377	0.28	$C_6H_{14}O_3$	134.17		2-Propanol, 1,1'-oxybis- **(newly identified)**	Dialkyl ether
16	5.456	0.15	$C_6H_{12}O_3$	132.16		1,3-Dioxolane-4-methanol, 2-ethyl- **(newly identified)**	Cyclic ether
17	6.9	0.10	$C_8H_6O_2$	134.13		1,2-Benzenedicarbox aldehyde	Aromatic aldehyde
18	7.074	0.59	$C_4H_6O_3$	102.08		2-Hydroxy-gamma-butyrolactone	Cyclic esters
19	7.254	0.25	C_3H_6O	58.08		2-Propen-1-ol	Alcohol
20	7.933	0.14	$C_7H_{12}O_2$	128.17		5H-1,4-Dioxepin, 2,3-dihydro-2,5-dimethyl- **(newly identified)**	Cyclic ether

21	8.792	0.11	$C_6H_{12}O_4$	148.16		2,3-Dihydroxy-3-methylpentanoic acid **(newly identified)**	Hydroxycarboxylic acid
22	9.928	0.61	C_8H_8O	120.15		Benzofuran, 2,3-dihydro-	Heterocyclic aromatic
23	10.109	0.23	$C_8H_{20}OSi$	160.33		1-Dimethyl(isopropyl) silyloxypropane **(newly identified)**	Organosilicon
24	10.235	0.23	$C_4H_{10}O_4$	122.12		Erythritol **(newly identified)**	Polyol
25	10.747	0.15	$C_6H_{10}O_3$	130.14		Ethyl 2,3-epoxybutyrate	Carboxylate ester
26	10.862	0.25	$C_4H_6O_3$	102.09		2-Hydroxy-gamma-butyrolactone	Hydroxy-substituted lactone
27	10.949	0.20	$C_9H_{10}O_2$	150.17		2-Methoxy-4-vinylphenol	Methoxyphenol
28	11.02	0.14	$C_9H_{18}O_3$	174.24		3-Methylbutan-2-yl propyl carbonate	Organic ester
29	11.355	0.20	$C_{10}H_{10}O_2$	162.19		Methyl cis-cinnamate **(newly identified)**	Ester
30	11.701	1.24	$C_{10}H_{10}O_2$	162.18		2-Propenoic acid, 3-phenyl-, methyl ester	Ester
31	11.77	0.11	$C_{13}H_{18}O_2$	206.28		1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta [c]pyran-1-yl) ethenone **(newly identified)**	Ketone
32	12.106	0.31	$C_{12}H_{22}O_{11}$	342.29		Sucrose	Disaccharide
33	12.41	0.12	$C_{13}H_{18}O$	190.28		3-(4-Isopropylphenyl)-2-methyl propionaldehyde **(newly identified)**	Aldehyde
34	12.688	0.47	$C_6H_{10}O_5$	162.14		beta.-D-Glucopyranose, 1,6-anhydro-	Anhydrohexose

35	13.824	0.16	$C_{12}H_{18}O_2$	194.27		6-Acetyl-4,4,7-trimethylbicyclo[4.1.0]heptan-2-one **(newly identified)**	Ketone
36	13.91	0.17	$C_9H_{20}O$	144.25		1-Heptanol, 2,4-dimethyl-, (R,R)-(+)- **(newly identified)**	Chiral alcohol
37	14.263	0.10	$C_{14}H_{18}O$	202.29		Cinnamaldehyde, .alpha.-pentyl- **(newly identified)**	Aldehyde
38	14.725	0.16	$C_{14}H_{20}O_3$	236.31		5,6,6-Trimethyl-5-(3-oxobut-1-enyl)-1-oxaspiro[2.5]octan-4-one **(newly identified)**	Ketone
39	15.016	0.11	$C_{15}H_{30}O_2$	242.39		Methyl tetradecanoate	Ester
40	15.535	0.32	$C_{25}H_{38}B_2O_2S$	424.3		Propane, 2-(9-borabicyclo[3.3.1]non-9-yloxy)-3-(9-borabicyclo[3.3.1]non-9-ylthio)-1-phenoxy- **(newly identified)**	Organoboron
41	15.589	0.50	$C_{14}H_{21}N$	203.32		1-[1-Methyl-1-(4-methyl-cyclohex-3-enyl)-ethyl]-1H-pyrrole **(newly identified)**	Heterocyclic organic
42	15.759	0.40	$C_{22}H_{36}O_2$	332.52		2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15-tetramethyl-, acetate, (E,E,E)- **(newly identified)**	Terpenoids
43	15.835	0.49	$C_{15}H_{26}O$	222.37		Humulane-1,6-dien-3-ol **(newly identified)**	Sesquiterpenoid alcohol
44	15.878	0.54	$C_{22}H_{38}$	302.54		Naphthalene, 1,1'-ethylidenebis [decahydro- **(newly identified)**	Organic
45	15.998	1.86	$C_{15}H_{26}O$	222.37		Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, [1R-(1.alpha.,2.alpha.,3.beta.,6.alpha.)]- **(newly identified)**	Terpenoid alcohol
46	16.103	2.53	$C_{19}H_{30}O_2$	290.4		Cyclopentanecarboxylic acid, 3-isopropylidene-, bornyl ester **(newly identified)**	Terpenoid ester

47	16.222	2.32	$C_{15}H_{24}$	204.36		Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl- **(newly identified)**	Alkene
48	16.281	3.08	$C_{19}H_{34}$	262.47		(4aS,5S,8aS)-5-Isopentyl-1,1,4a,6-tetramethyl-1,2,3,4,4a,5,8,8a-octahydronaphthalene **(newly identified)**	Sesquiterpenoid
49	16.348	1.88	$C_{15}H_{24}O_3$	252.35		Ageratriol **(newly identified)**	Terpenoid
50	16.421	4.16	$C_{14}H_{24}O$	208.34		2-Buten-1-ol, 2-ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)- **(newly identified)**	Terpenoid
51	16.518	2.67	$C_{20}H_{38}$	278.52		Neophytadiene **(newly identified)**	Terpenoid
52	16.611	0.76	$C_{18}H_{36}O$	268.48		2-Pentadecanone, 6,10,14-trimethyl-	Ketone
53	16.697	1.91	$C_{20}H_{34}O$	290.48		(E)-3-Methyl-5-((1R,4aR,8aR)-5,5,8a-trimethyl-2-methylenedecahydronaphthalen-1-yl)pent-2-en-1-ol **(newly identified)**	Terpenoid
54	16.829	2.36	$C_{15}H_{24}$	204.35		Bicyclo[4.3.0]nonane, 7-methylene-2,4,4-trimethyl-2-vinyl- **(newly identified)**	Terpenoid
55	16.908	2.14	$C_{20}H_{34}O$	290.48		Thunbergol	Terpenoid
56	16.978	3.41	$C_{20}H_{34}O_2$	306.48		4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)- **(newly identified)**	Terpenoid
57	17.096	2.00	$C_{13}H_{17}F_5O_2$	300.26		Borneol, pentafluoropropionate	Ester
58	17.145	0.54	$C_{20}H_{40}$	280.53		2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, [R-[R*,R*-(E)]]- **(newly identified)**	Ester

59	17.258	0.17	$C_{15}H_{24}O$	220.35		Humulenol-II **(newly identified)**	Terpeniod
60	17.427	0.18	$C_{37}H_{65}NO_4Si_3$	672.2		Pregnan-20-one, 3,11,21-tris[(trimethylsilyl)oxy]-, O-(phenylmethyl)oxim, (3.beta.,5.alpha.,11.beta.)- **(newly identified)**	Steroid
61	17.591	0.14	$C_{18}H_{52}O_7Si_7$	577.2		3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane **(newly identified)**	Siloxane
62	17.835	1.95	$C_{17}H_{34}O_2$	270.45		Hexadecanoic acid, methyl ester	Fatty acid, ester
63	18.887	0.15	$C_{17}H_{34}O_2$	270.45		Pentadecanoic acid, ethyl ester	Fatty acid, ester
64	20.564	0.87	$C_{19}H_{34}O_2$	294.47		9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Fatty acid, ester
65	20.669	1.94	$C_{19}H_{32}O_2$	292.46		9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Fatty acid, ester
66	20.823	3.93	$C_{20}H_{40}O$	296.53		Phytol	Terpenoid
67	21.075	0.99	$C_{19}H_{38}O_2$	298.50		Methyl stearate	Ester
68	21.678	0.17	$C_{21}H_{38}O_2$	322.5		[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester (newly identified)	Carboxylic acid, ester
69	21.769	0.18	$C_{20}H_{34}O_2$	306.48		9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	Fatty acid, ester
70	22.017	0.12	$C_{14}H_{29}NO$	227.39		Tetradecanamide	Amide
71	24.117	0.11	BD			Dimethyl *u-truxinate	Ester
72	25.055	3.27	$C_{18}H_{35}NO$	281.48		9-Octadecenamide, (Z)-	Amide
73	25.235	0.12	$C_{20}H_{34}O_2$	306.5		(1S,2E,4S,5R,7E,11E)-Cembra-2,7,11-trien-4,5-diol **(newly identified)**	Terpenoid
74	25.696	0.12	$C_{20}H_{42}$	282.55		Eicosane	Alkane
75	27.291	0.09	$C_{30}H_{61}I$	548.71		Triacontane, 1-iodo-	Alkane
76	27.68	0.10	$C_{27}H_{54}O_2$	410.72		Hexacosanoic acid, methyl ester	Fatty acid, ester
77	27.764	0.18	$C_{24}H_{38}O_4$	390.56		Bis(2-ethylhexyl) phthalate	Ester
78	28.57	0.11	$C_{18}H_{54}O_9Si_9$	667.39		Cyclononasiloxane, octadecamethyl- **(newly identified)**	Siloxane

79	31.984	0.88	$C_{30}H_{50}$	410.7180		Squalene	Triterpene
80	33.895	1.16	$C_{27}H_{46}O_2$	402.65		.delta.-Tocopherol	Vitamin E
81	35.128	0.32	$C_{28}H_{48}O_2$	416.6795		(R)-6-Methoxy-2,8-dimethyl-2-((4R,8R)-4,8,12-trimethyltridecyl)chroman **(newly identified)**	Flavonoid
82	35.377	0.26	$C_{28}H_{48}O_2$	416.68		.gamma.-Tocopherol	Vitamin E
83	36.549	0.16	$C_{29}H_{50}O_2$	430.71		Vitamin E	Vitamin E

Among the identified bioactive components, thioglycolic acid has highest percent peak area. This compound is used as a reducing agent.[37] 9, 12, 15 Octadecatrien-1-ol (Z, Z, Z) exhibited antioxidant and antibacterial properties.[38] n-Hexadecanoic acid showed a range of biological activities, including antioxidant, 5-alpha reductase inhibition, anti-fibrinolytic, hemolytic, antimicrobial, hypocholesterolemic, nematicidal, pesticidal, and antiandrogenic effects. Additionally, it contributes to flavor and possesses hemolytic properties.[39] Anti-inflammatory, cancer-preventive, hepatoprotective, antioxidant, and hypocholesterolemic properties are all indicated by methyl 9,12,15-octadecatrienoate (Z,Z,Z).[40] The other main volatile substances that are present include phenolic compounds, esters, alkanes, aldehydes, alkenes, and ketones. These substances have cytotoxic, anti-inflammatory, anti-arthritic, antidiabetic, hypolipidemic, and antiulcer properties. Antioxidant, neuroprotective, antibacterial, anticancer, anti-inflammatory, and anti-diuretic properties have been described for phytol.[41] 9,12-Octadecadienoic acid, methyl ester inhibits 5-alpha-reductase, has anti-inflammatory, anti-arthritic, hepatoprotective, antiandrogenic, hypocholesterolemic, nematicide, antihistaminic, anticoronary, insectifuge, antieczemic, and antiacne effects.[42] Erythritol has vasoprotective effect and antioxidant activity [43]. Anticancer, antioxidant, drug carrier, hypocholesterolemic, detoxifying, skin hydrating, and emollient activities of triterpenes have been reported.[44] These bioactive phytochemicals are the basis of therapeutic potential of medicinal plants and useful in the treatment of several diseases. The medicinal significance of the plant is because of the bioactive phytochemical compounds that generate characteristics physiological action on humans.

Conclusion

A total of 83 compounds were identified from the GC-MS analysis of methanol extract of *A. carambola* leaves exhibiting various phytochemical activities. Among these, 40 compounds were reported for the first time. The compounds are responsible for the different therapeutic and pharmacological properties. Previous studies showed that the plant exhibits important pharmacological activities including anti-inflammatory, anti-microbial, anti-oxidant, analgesic, anti-diabetic and anti-diarrheal properties. For the potential creation of novel medicinal products utilizing some of the bioactive substances present in of *A. carambola*, more research is necessary.

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Author contributions

Mahmuda Nasrin prepared the plant extraction and conducted the manuscript. Farjana Afrin carried out the phytochemical analysis, while Faria Tasnif Alin contributed to the GC-MS analysis.

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