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Chemical and biological activity studies of *Averrhoa carambola* L.

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ABSTRACT

Averrhoa carambola L., belonging to the Oxalidaceae family is one of the popular fruits in Bangladesh. The objective of the study is to isolate and purify secondary metabolites from *A. carambola* leaves and investigate phytochemical properties, fatty acid, antioxidant, antimicrobial activity, and cytotoxicity in both fruits and leaves. Phytochemical screening revealed the presence of lignins, quinones, saponins, flavonoids, phenol, carbohydrates, steroids, and terpenoids. Repeated column chromatography of the dichloromethane (DCM) extract of leaves yielded three compounds: β-sitosterol, 6,9-dieneoctadeca-1-ol, and palmitic acid, characterized using spectroscopic techniques. The n-hexane extract of the fruit showed the presence of palmitoleic acid (20.94%) and octadecanoic acid (48.48%), while palmitoleic acid (6.47%), octadecanoic acid (23.23%), and behenic acid (41.70%) predominated in the leaves. For DPPH free radical scavenging activity, the IC_{50} value of methanol (MeOH) extracts was found to be 16.71 and 27.30 µg/mL in fruits and leaves, respectively, which is lower than that of n-hexane (IC_{50} value 282.46 and 78.65 µg/mL in fruits and leaves, respectively) and DCM (IC_{50} value 32.98 and 39.38 µg/mL in fruits and leaves, respectively) extracts. None of the extracts showed significant antibacterial or antifungal activities. Cytotoxicity assay was performed on HeLa and Vero cell lines, and only the DCM extract of fruits exhibited cytotoxicity on the Vero cell line.

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1. Introduction

Medicinal plants possess therapeutic value in any of their parts, including leaves, stems, barks, roots, flowers, or fruits, which are used to prepare medicine or as precursors for the production of pharmaceutical drugs. Plants have the ability to synthesize a wide variety of chemical compounds used to perform important biological functions, including defense against insects, fungi, diseases, and herbivorous mammals (Cushnie et al., 2020). *Averrhoa carambola* L., belonging to the Oxalidaceae family and locally known as Kamranga in Bangladesh, is a medium-sized tree. The species in the genus Averrhoa originated in Ceylon and the Moluccas (Jabbar et al., 1995), but it has been cultivated in Southeast Asia, Malaysia, and Bangladesh for hundreds of years (Nandkarni, 1976). It is fully packed with vital nutrients and is a good source of natural antioxidants like L-ascorbic acid, (-) epicatechin, and gallic acid in gallotannin forms (Chang et al., 2002), as well as folate and pantothenic acid. Despite lacking scientific proof to ensure the phytochemical and antimicrobial activity, it is commonly used for treating various ailments in many societies. Fruits and juice of *A. carambola* are used as astringents and tonics to treat diarrhea, vomiting, dysentery, hepatic colic, bleeding piles, relieving thirst and febrile excitement. Leaves are antipruritic, antipyretic, anthelmintic, and are also useful in treating scabies, fractured bones, various types of poisoning, intermittent fevers, and intestinal worms (Vasconcelos et al., 2005). Fatty acids have diverse functions in cells, ranging from structural "building blocks" of cell membranes to suppliers of energy and signaling molecules. Fatty acids may play a positive role in our biological system, and the fatty acid profile contributes to enriching knowledge for further advanced research in the field of phytochemistry (de Carvalho et al., 2018). Phenolic compounds are common plant secondary metabolites that have physiological functions in plants and positive effects on human health, as they can act as antioxidants. Antioxidants play important roles in preventing pathogenic processes related to cancer, cardiovascular disease, macular degeneration, cataracts, and asthma, and can enhance immune function (alişkan et al., 2011). Antioxidant defenses protect the body from the detrimental effects of free radicals generated as by-products of normal metabolism (Nakilcioğlu et al., 2013). Previous phytochemical investigations led to the isolation of 5-hydroxymethyl-2-furfural (Macleod et al., 1990; Frohlich et al., 2006), L-ascorbic acid, (-)-epicatechin, and gallic acid (Shui et al., 2004), as well as dihydroabscissic alcohol from*A. carambola* (Lutz et al., 1994). This paper reports the isolation and structure elucidation of three secondary metabolites characterized by spectroscopic techniques, including UV-Vis, FT-IR, ^{1}H and ^{13}C NMR, and investigates the fatty acid profiles, DPPH

free radical scavenging activity, antimicrobial activity, and cytotoxicity assay of *A. carambola*.

2. Materials and methods

2.1. Sample collection

A. carambola fruits were collected from the local market, and leaves were collected from Khulna district, Bangladesh. Fresh fruits were washed with water, excess water was wiped off, then chopped into small pieces of nearly the same diameter, and dried in open air. Leaves were also dried in open air. Finally, both dried fruits and leaves were ground with a grinding machine to become a powder form (30 meshes).

2.2. Extraction procedures

The extraction process was conducted for both leaf and fruit samples using a successive method from non-polar (n-hexane) to more polar (MeOH) solvents to ensure the extraction of a wide range of compounds. Dried leaf (411.0 g) and fruit (80.0 g) powder samples were successively extracted with n-hexane, DCM, and MeOH at room temperature. The extracts were dried under reduced pressure using a rotary evaporator at 40 to 50°C, resulting in the three extracts from the fruits and leaves of *A. carambola*, as shown in Table 1.

Table 1. Amount of different extracts obtained from *A. carambola*

Parts of *A. carambola*	n-Hexane (g)	DCM (g)	MeOH (g)
Leaves	10.0	12.0	74.0
Fruits	1.0	2.0	7.0

2.3. Antibacterial activity

n-Hexane, DCM, and MeOH extracts of *A. carambola* fruits and leaves dissolved in 2.5% DMSO solvent were subjected to antimicrobial screening with a concentration of 6 µg/disc in every case. The antibacterial screening of different solvent extracts was determined by adopting the disk-diffusion method (Razmavar et al., 2014) for *Bacillus cereus* and *Escherichia coli* bacteria using Mueller Hinton Agar medium composed of beef infusion (0.30 g), casamino acid (1.75 g), starch (0.15 g), Bacto agar (1.70 g), distilled water (100 mL), and pH (7.3 at 25°C).

2.4. Antifungal activity

The antifungal activity of n-Hexane, DCM, and MeOH extracts of *A. carambola* fruits and leaves (5 mg/2 mL in 2.5% DMSO) were tested against two fungi (*A. niger and Candida sp.*). The antifungal activity of different solvent extracts was determined by the well diffusion method (Balouiri et al., 2016). A spore suspension of *A. niger* and *Candida sp.* was prepared in sterile 0.85% NaCl with Tween 20 (1%) from fresh colonies grown on Sabouraud dextrose agar at 35°C for 5 days. The cell concentration was adjusted to final concentrations of 0.4 x 10^1 to 5 x 10^1 CFU/mL. These suspensions were used directly for inoculation. The antifungal activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeters using a transparent scale. Each assay was performed in triplicate and on three different days, and the mean diameters are reported.

2.5. Cytotoxicity assay on cancer cell line

The cytotoxicity assay of *A. carambola* extracts (5 mg/2 mL in 2.5% DMSO) was assessed against a human cervical carcinoma cell line, HeLa, which was maintained in DMEM (Dulbecco's modified Eagle's medium) containing 1% penicillin-streptomycin (1:1), 0.2% gentamicin, and 10% fetal bovine serum (FBS). Cells (4×10^4/200 µL) were seeded onto a 48-well plate and incubated at 37°C + 5% CO_2. The next day, 50 µL of the sample (filtered) was added to each well. Samples were dissolved in 2.5% DMSO, and duplicate wells were used for each sample (Saroar et al., 2020). An *in vitro* cytotoxicity test was performed on HeLa cell line (a human cervical carcinoma cell) and Vero cell line (Kidney epithelial cells extracted from an African green monkey).

2.6. Isolation of compounds by column chromatography

The dried DCM leaf extract (12.0 g) was mixed with column-grade silica gel. The sample was placed on the top of the bed of the column packed with column-grade silica gel. The column was first eluted with 100% n-hexane and then eluted with mixtures of n-hexane with an increasing amount of DCM and finally eluted with MeOH. All the column fractions were screened by TLC under UV light and by spraying with the vanillin-H_2SO_4 reagent. Depending on the TLC pattern, similar fractions were combined together, and one fraction gave a single spot on the TLC plate, collected from 90% DCM extracts in n-hexane and was purified by washing with n-hexane several times to obtain compound **1** (5.6 mg). Then 40% DCM in n-hexane fraction and 60% DCM in n-hexane were further carried by a sub-column, and compound **2** and compound **3** were obtained from 40% DCM in n-hexane (70 mg) and 60% DCM in n-hexane (4.2 mg), respectively.

2.7. Fatty acid composition

Approximately 200 mg of *A. carambola* fruit n-hexane extract was taken in a pear-shaped flask, and 10.0 mL of 0.5 M methanolic NaOH was added to it. The mixture was ultrasonicated and refluxed for 40 min. The mixture was evaporated with a rotavapour to dryness and dissolved in water (pH 4.5). The mixture was transferred into a separatory funnel and shaken vigorously. Then extracted with n-Hexane (25 mL × 2). The organic layer was collected and evaporated to dryness. Exactly 3.0 mL of Boron trifluoride-MeOH mixture was added to it and ultrasonicated for 1 min. The mixture was refluxed at a boiling water bath for 20 min. The acetylated mixture was evaporated to dryness. After that, 3.0 mL of n-Hexane was added to the flask, the mixture was ultrasonicated for 1 min. Then it was filtered through a cotton filter by a Pasteur pipette with anhydrous Na_2SO_4 and transferred into a GC vial for analysis by GC-FID. The same procedure was followed for *A. carambola* leaves extract (n-hexane).

A GC Shimadzu (GC-2025) Gas Chromatograph having an FID detector was used for the identification and quantification of fatty acids. Separations were performed on WCOT quartz capillary (DB-5) column (30 m in length and 0.25 mm in diameter). The temperature program in the oven was as follows: 120°C for 1 min (hold), then increased by 7°C/min to 280°C and held for 6 min. N_2 was used as the carrier gas with a column flow rate of 2 mL/min. The injected volume was 1.0 µL. Column, injector, and detector temperature were set at 270, 280, and 290°C respectively. Air and nitrogen gases were used as fuel for FID.

Methyl ester of thirteen fatty acid standards of the highest available purity (99.9%) were purchased from Sigma (USA). The standards were stored in the freezer at -20°C. Fatty acids present in *A. carambola* (both fruits and leaves) samples were converted to their corresponding methyl esters to make them volatile and easily separated into individual components in a gas chromatographic condition (Shoeb et al., 2013). Fatty acids were identified by comparing their retention time with standard fatty acid chromatogram. The equation used for the determination of the percentage of fatty acid is:

$$\text{Fatty acid (\%)} = \frac{\text{Peak area of particular fatty acid}}{\text{Total peak area of all fatty acids}} \times 100$$

2.8. Antioxidant activity (DPPH assay method)

DPPH free radical scavenging activity was evaluated using ascorbic acid as a standard (Jayaprakasha et al., 2000; Saroar et al., 2020). A stock solution (1 mg/mL) of the different solvent fractions of A. carambola (both fruits and leaves) was prepared in the respective solvent systems, from which serial dilutions were carried out to obtain concentrations of 5, 10, 20, 40, 60, 80, 100, 250, 500 μg/mL. In this assay, 2 mL of 0.1 mM DPPH solution was added to 2 mL of extract solution at different concentrations, and the contents were stirred vigorously for 15 s. Then the solutions were allowed to stand in a dark place at room temperature for 30 min for the reaction to occur. After 30 min, absorbance was measured against a blank at 517 nm using a spectrophotometer. The percentage of DPPH radical-scavenging activity of each plant extract was calculated as:

$$\text{DPPH radical-scavenging activity (I \%)} = \left[\frac{(A_o - A)}{A_o}\right] \times 100$$

Where, A_o = absorbance of the control solution (containing all reagents except plant extract) and A = absorbance of the DPPH solution containing plant extract.

3. Results and discussion

The phytochemical screening of the extracts of *A. carambola* revealed the presence of lignins, quinones, saponins, flavonoids, phenol, carbohydrates, steroids, and terpenoids. Through repeated column chromatography of the DCM extract of *A. carambola* leaves, three secondary metabolites were obtained, which were characterized as β-sitosterol (compound **1**), 6, 9-dieneoctadeca-1-ol (compound **2**), and palmitic acid (compound **3**), using spectroscopic techniques such as FT-IR, ^{1}H NMR, and ^{13}C NMR, and were compared with reported data. The DCM extract was preferred for compound isolation as it contains a more diverse range of organic compounds that are relatively easy to isolate by using normal phase column chromatography than n-hexane and MeOH as extractive solvents.

Compound 1: The compound 1 (5.6 mg) appeared as white crystals with an R_f value of 0.65 (n-hexane: DCM-10:90), and its melting point was found to be 158-160°C. The FT-IR spectrum of compound **1** showed absorption bands at 3437, 2942, 1610, 1455, 1372, 1060, 964, and 669 cm^{-1} due to O-H (stretching), sp^3 C-H (stretching), >C=C (stretching), >CH_2 (bending), -CH_3 (bending), C-O (stretching), Csp^3-H (out-of-plane bending), and C-H (bending), respectively. The ^{1}H NMR (Bruker, 400 MHz, $CDCl_3$) spectrum of the isolated compound **1** had two sharp singlets (*s*) at δ 0.67 and 1.00 ppm, typical for the presence of methyl protons (H-18 and H-19) at C-10 and C-13, respectively. The spectrum had a multiplet at δ 3.52 ppm indicative of the presence of an oxymethine proton (H-3). The distorted multiplet at δ 5.35 ppm was indicative of the presence of an olefinic proton (H-6) at C-6. The three doublets at δ 0.85, 0.83, and 0.81 ppm were due to the presence of methyl protons at C-21, C-26, and C-27, respectively. The spectrum had a triplet at δ 1.29 ppm due to the presence of a methyl proton at C-29. The spectrum had a broad singlet at δ 1.83 ppm, which was due to the presence of a hydroxyl proton (OH-3) at C-3. The other signals of the spectrum between 1.42–1.98 ppm were due to the presence of different methylene (-CH_2-) and methine (>CH-) protons. The ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **1** showed a chemical shift δ at 37.2 (C-1), 31.7 (C-2), 71.8 (C-3), 42.3 (C-4), 140.7 (C-5), 121.7 (C-6), 31.9 (C-7), 31.6 (C-8), 50.1 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 56.7 (C-14), 23.1 (C-15), 28.2 (C-16), 56.0 (C-17), 12.0 (C-18), 19.8 (C-19), 36.1 (C-20), 18.8 (C-21), 33.9 (C-22), 26.1 (C-23), 45.8 (C-24), 29.4 (C-25), 19.4 (C-26), 19.8 (C-27), 24.3 (C-28), and 11.8 (C-29) ppm. The ^{13}C NMR spectrum (Bruker, 100 MHz, $CDCl_3$) of isolated compound **1** showed the presence of 29 carbon signals. Among them, 2 signals were assignable to sp^2 carbons, 11 signals to methylene carbons, 8 signals to methine carbons, 6 signals to methyl carbons, and 2 signals for quaternary carbons. The signals at δ 140.7 and 121.7 ppm were due to olefinic carbons, and signals at δ 42.3 and 36.5 ppm were assignable to two quaternary carbons. The signals at δ 71.8 ppm were due to oxymethine carbon. From the physical characteristics and spectral analysis (^{1}H NMR and ^{13}C NMR) data of compound **1** and comparing the reported data (Pateh et al., 2008) of ^{1}H and ^{13}C NMR spectral data of β-sitosterol, the structure of the compound was established as β-sitosterol, as shown in **Fig. 1**. β-sitosterol was isolated from the n-hexane extract of *A. carambola* leaves in some previous studies (Moresco et al., 2012, De-Eknamkul et al., 2003).

Compound 2: Compound **2** (70 mg) was a yellow semi-solid with an R_f value of 0.58 (n-hexane: DCM-40:60). The FT-IR spectrum of compound **2** exhibited absorption bands at 3437 (O-H, stretching), 2942 (Csp^3-H, stretching), 1610 (>C=C, stretching), 1455 (>CH_2, bending), 1372 (-CH_3, bending), 1060 (C-O, stretching), 964 (sp^2 C-H, out-of-plane bending), and 669 (C-H, bending) cm^{-1}. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound 2 displayed signals at δ 5.35 ppm for two olefinic protons at C-6 and C-7, as well as C-9 and C-10. A distorted triplet at δ 0.94 ppm indicated a methyl group. A strong, sharp peak at δ 1.28 ppm was due to the presence of protons at C-3, C-4, and C-12 to C-17. Signals at δ 2.07 were attributed to protons at C-6, C-7, and C-11. The band at δ 3.64 ppm corresponded to the methylene proton next to the oxygen of –OH at C-1 (H-1), and at δ 1.34 ppm, a signal for the –OH proton at C-1 was observed. The ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **2** exhibited signals at δ 135.3, 63.1, 32.8, 32.2, 29.7, 29.6, and 14.2 ppm, indicating the presence of 18 carbons. The signal at δ 63.13 ppm corresponded to the alcoholic carbon (>CH-OH) at C-1. The signal at δ 32.8 ppm was assigned to the methylene carbon attached to C-1. The signals at δ 29.7 ppm and 29.6 ppm were attributed to the methylene carbons at C-3 and C-4, respectively. The signal at δ 32.2 ppm was assigned to C-5, C-8, and C-11. A strong, sharp peak at δ 135.3 ppm indicated the presence of a double bond between C-6 and C-7, as well as C-9 and C-10. The signal at δ 29.7 indicated the presence of a methylene group at C-12 to C-17. The signal at δ 14.2 ppm indicated the presence of a methyl group. Based on the physical characteristics and spectral analysis (^{1}H and ^{13}C NMR) data of compound **2**, and comparing it with the reported data (Miyake et al., 1998) of ^{1}H NMR and ^{13}C NMR spectral data of 6,9-dieneoctadeca-1-ol, the structure of the compound was identified as 6,9-dieneoctadeca-1-ol, as shown in Fig. 1.

Compound 3: Compound **3** (4.2 mg) appeared as a yellow semi-solid with an R_f value of 0.55 (n-hexane: DCM-60:40), and its melting point was determined to be 61.63°C. The FT-IR spectrum of compound **3** exhibited absorption bands at 3447 (O-H, stretching), 2922 (sp^3 C-H, stretching), 1737 (>C=O, stretching), 1455 (>CH_2, bending), 1372 (-CH_3, bending), 1173 (C-O, stretching), and 676 (C-H, bending) cm-1. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of the isolated compound **3** displayed a peak at δ 0.88 ppm indicating the presence of three protons from a methyl group. A strong, sharp peak at δ 1.33 ppm corresponded to the methylene protons at H-5 to H-15. The signals at δ 2.30, 1.56, and 1.29 ppm were attributed to the methylene protons of H-2, H-3, and H-4, respectively. The band at δ 9.76 ppm was assigned to the

C-1 proton (-COOH). The other peak in the ^{1}H NMR spectrum was attributed to impurities. The ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of the isolated compound **3** exhibited signals corresponding to 16 carbons. The signal at δ 34.4 ppm was assigned to the methylene carbon attached to C-1. The signals at δ 24.8 and 23.4 ppm corresponded to the methylene carbons at C-3 and C-4, respectively. The intense signal at δ 29.5 ppm indicated a long chain $-CH_2-$, which encompassed C-5 to C-13. The signals at δ 31.9 and 22.7 ppm were attributed to the methylene carbons at C-14 and C-15, respectively. The signal at δ 14.1 ppm was assigned to the methyl carbon at C-16. Based on the comparison of ^{1}H NMR and ^{13}C NMR values with reported data (Miyake et al., 1998), the structure of compound **3** was confirmed as palmitic acid, as shown Fig. 1. Palmitic acid was also identified in both the leaves and fruits extracts of *A. carambola* by Wei et al (2014).

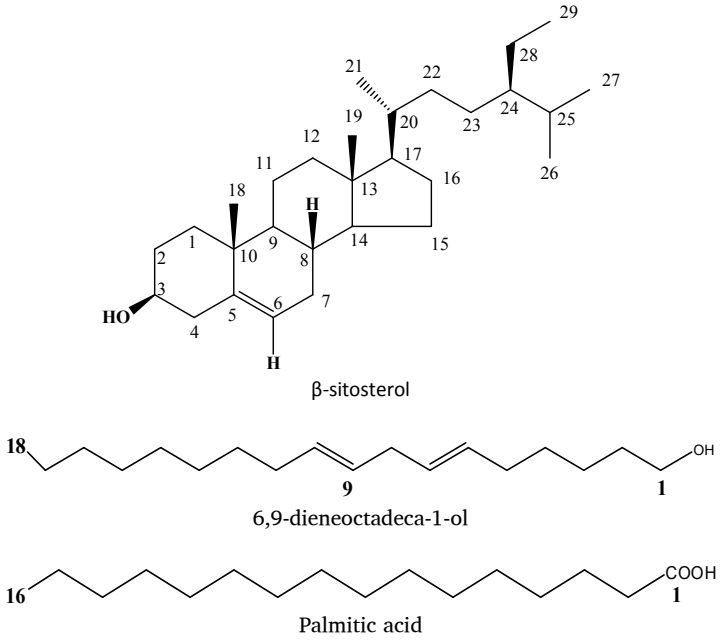


Fig. 1. Structure of compounds

3.1. Fatty acid analysis

The n-hexane extracts of leaves and fruits were analyzed to determine their fatty acid composition using GC-FID. The analysis revealed that *A. carambola* fruits contained the highest proportion of octadecanoic acid (48.48%), while tetradecanoic acid was present in the lowest proportion (0.47%). Other fatty acids such as palmitic, palmitoleic, linoleic, and behenic acids were present at 0.84%, 20.94%, 5.09%, and 1.04%, respectively. Among the fatty acids, behenic acid was the most abundant (41.70%) in *A. carambola* leaves, while palmitic, palmitoleic, octadecanoic, and linoleic acids were present at 1.78%, 6.47%, 23.23%, and 3.52%, respectively (Tasnim et al., 2020).

3.2. Free radical scavenging activity

The percentage of DPPH free radical scavenging activity and the IC_{50} values of different extracts of *A. carambola* are presented in Table 2. The IC_{50} values were found to be 282.46 µg/mL for n-hexane, 32.98 µg/mL for DCM, and 16.71 µg/mL for MeOH extracts in *A. carambola* fruits. Similarly, for *A. carambola* leaves, the IC_{50} values were 78.65 µg/mL for n-hexane, 39.38 µg/mL for DCM, and 27.30 µg/mL for MeOH extracts. Samples with IC_{50} values ranging from 50 to 100 µg/mL are considered to exhibit intermediate antioxidant activity (Phongpaichit et al., 2007), while samples with IC_{50} values ranging between 10 to 50 µg/mL are considered to possess strong antioxidant activity.

Based on these results, it can be recommended that the MeOH extract of *A. carambola* (both fruits and leaves) possesses high antioxidant capacity. Synthetic antioxidants may contain impurities and carry the risk of overdosage, with potential carcinogenic effects. In contrast, natural antioxidants are safer and purer. Therefore, the leaves of *A. carambola* can serve as a source of natural antioxidants, contributing to improved reproductive function, immune system support, enhanced body defense mechanisms, optimal kidney function, and the maintenance of healthy vision.

3.3. Antibacterial activity

n-Hexane, DCM, and MeOH extracts of *A. carambola* fruits and leaves were dissolved in 2.5% DMSO and subjected to antimicrobial screening with a concentration of 6 µg/disc in each case. According to previous findings, the antimicrobial activities *of A. carambola* are concentration-dependent (Jorgensen et al., 2009). Since the experiment was conducted at the same concentration, none of the extracts showed any activity against the tested microbes. The zones of inhibition for each extract are presented in Fig. 2.

It was reported that the 70% ethanol extract of *A. carambola* fruits at 30 mg/mL exhibited antibacterial activity, showing inhibitions of 21 mm against *Salmonella typhi*, 20 mm against *Escherichia coli*, 19 mm against *Shigella boydii*, with the highest inhibition zones observed against *Salmonella typhi* and *Staphylococcus aureus* (21 mm each) (Maw and Thin Wa., 2017). The concentration used in this experiment is much lower than the reported data, which explains the lack of antibacterial activity.

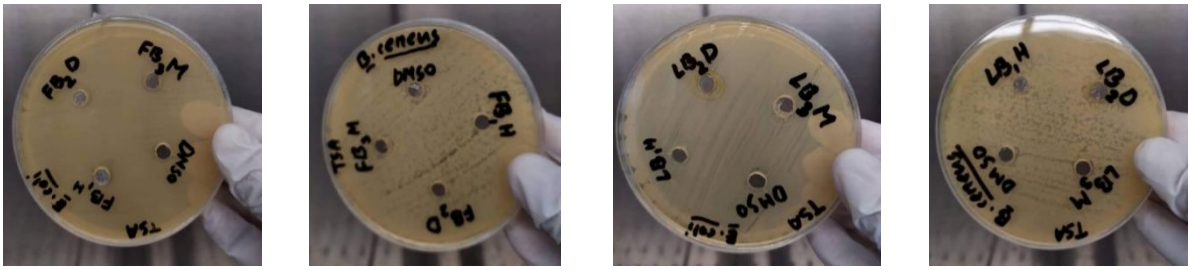
Table 2. DPPH radical activity of different extracts and standard tert-butyl-1-hydroxy toluene

	Extracts	% inhibition at different concentration (g/mL)									IC_{50} (g/mL)
		5	10	20	40	60	80	100	250	500	
Fruit	Hexane	12.1	15.1	35.2	36.2	36.9	37.1	39.7	47.3	62.7	282.46
	DCM	18.4	31.4	44.3	52.6	54.8	60.9	71.3	73.9	74.3	32.98
	MeOH	20.1	41.0	54.1	65.6	69.1	71.5	73.7	74.5	77.3	16.71
Leaf	Hexane	8.8	11.2	19.6	30.2	36.9	51.3	63.8	65.9	68.9	78.65
	DCM	8.4	12.3	22.9	51.0	53.8	62.1	72.5	73.9	80.1	39.38
	MeOH	3.7	5.2	30.9	75.6	76.1	79.8	83.2	92.5	96.1	27.30
	BHT	64.2	90.2	92.5	93.5	93.8	95.1	95.7	98.2	99.5	3.68

3.4. Antifungal activity

The n-hexane, DCM, and MeOH extracts of *A. carambola* fruits and leaves did not show any activity against A. niger and *Candida sp.* The zones of inhibition for each extract against *Candida sp.* and *A. niger* are displayed in Fig. 3 and Fig. 4, respectively.

Irianti reported that a CV assay was employed to determine *A. carambola*'s ability to suppress the growth of *Candida albicans*. According to the decrease in optical density (OD), the ethyl acetate extract of *A. carambola* leaves exhibited considerable suppression of Candida development. Roughly 30 mg of *A. carambola* ethyl acetate fractions were dissolved in 100 µl of DMSO. Cell growth was substantially inhibited by over 60% when the ethyl acetate extract of *A. carambola* leaves was added. Thin-layer chromatography results revealed that the leaves of *A. carambola* contained flavonoids and steroids (Irianti et al., 2022).



(a) (b) (c) (d)

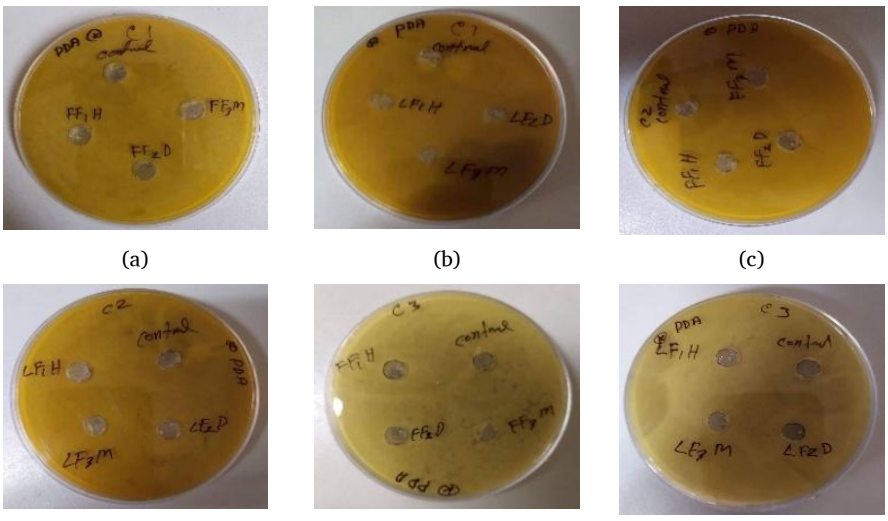
Fig. 2. Antibacterial activity of different extracts of *A. carambola* against bacteria (FB_1H, FB_2D, FB_3M-hexane, DCM and MeOH extracts of fruit, respectively and LB_1H, LB_2D, LB_3M- hexane, DCM and MeOH extracts of leaves, respectively)

3.5. Cytotoxicity of *A. carambola*

The n-hexane, DCM, and MeOH extracts of *A. carambola* fruits and leaves, dissolved in 2.5% DMSO solvent, were investigated for their cytotoxic effects against Hela (human cervical) and Vero (kidney epithelial) cell lines. The results are presented in the Table 3.

As the DCM extract of the fruit exhibited cytotoxicity on the Vero cell line, indicating the presence of cytotoxic compounds in that extract, *A. carambola* fruits could be explored for developing anti-cancer therapeutic agents. However, this study provides only preliminary data, and further studies are required for the isolation and identification of biologically active substances from these extracts. Cytotoxicity was observed for the DCM extract of the fruit on the Vero cell line. Images of all extracts for the Vero cell and Hela cell lines are provided in Fig. 5 and Fig. 6, respectively.

A report states that hepatocellular carcinoma (HCC), the most prevalent form of liver cancer, remains one of the deadliest cancers globally. In mice exposed to carcinogens, tumor occurrence was zero percent, while administration of *A. carambola* extracts significantly reduced tumor incidence, yield, and burden. As the second leading cause of global mortality, cancer presents a significant challenge. *A. carambola* extract (99% ethyl alcohol extract) protected mice from the negative physical and biochemical changes induced by DENA/CCl_4 during hepatocarcinogenesis (Singh et al., 2014).

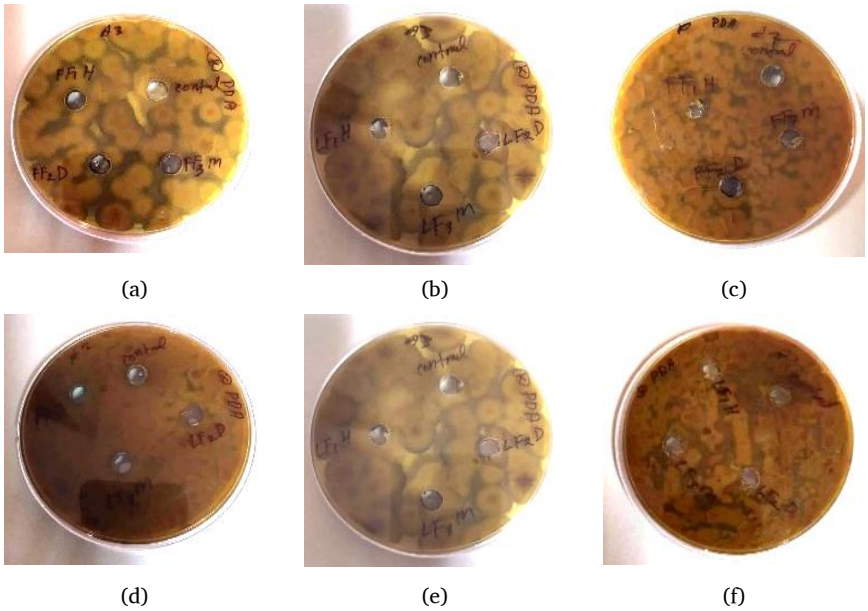


(a) (b) (c) (d) (e) (f)

Fig. 3. Antifungal activity of different extracts of *A. carambola* against *Candida sp.* (FF_1H, FF_2D, FF_3M-hexane, DCM and MeOH extracts of fruit, respectively and LF_1H, LF_2D, LF_3M- hexane, DCM and MeOH extracts of leaves, respectively)

Table 3. Cytotoxicity of different extracts of *A. carambola* using 2.5% DMSO

Sample		Solvent (-)	Solvent (+)	Fruits extracts n-Hexane	DCM	MeOH	Leaves extracts n-Hexane	DCM	MeOH
Survival of cells	HeLa	100%	>95%	>95%	>95%	>95%	>95%	>95%	>95%
	Vero	100%	>95%	>95%	40-50%	>95%	>95%	>95%	>95%



(a) (b) (c)

(d) (e) (f)

Fig. 4. Antifungal activity of different extracts of *A. carambola* against *A. niger* (FF_1H, FF_2D, FF_3M-hexane, DCM and MeOH extracts of fruit, respectively and LF_1H, LF_2D, LF_3M- hexane, DCM and MeOH extracts of leaves, respectively).

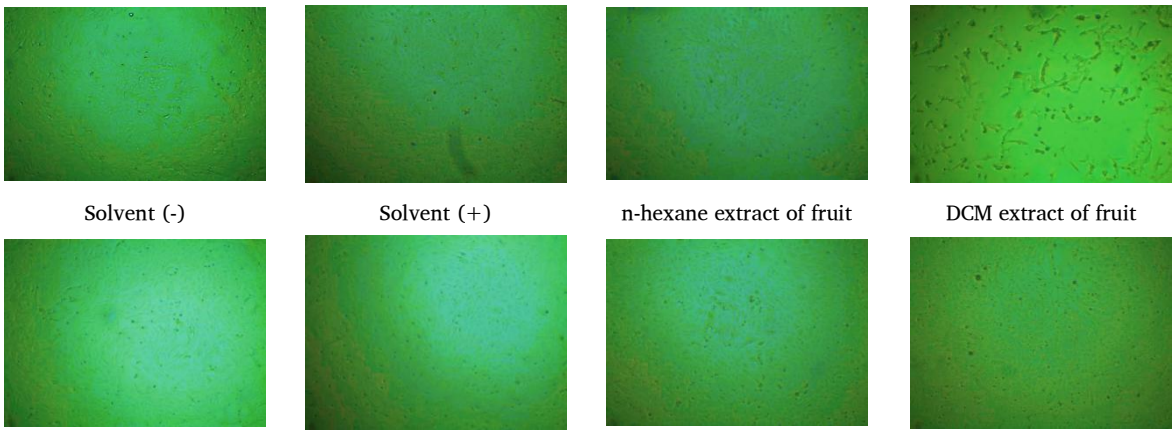


Fig. 5. Cytotoxicity of different extracts of *A. carambola* on Vero cell line

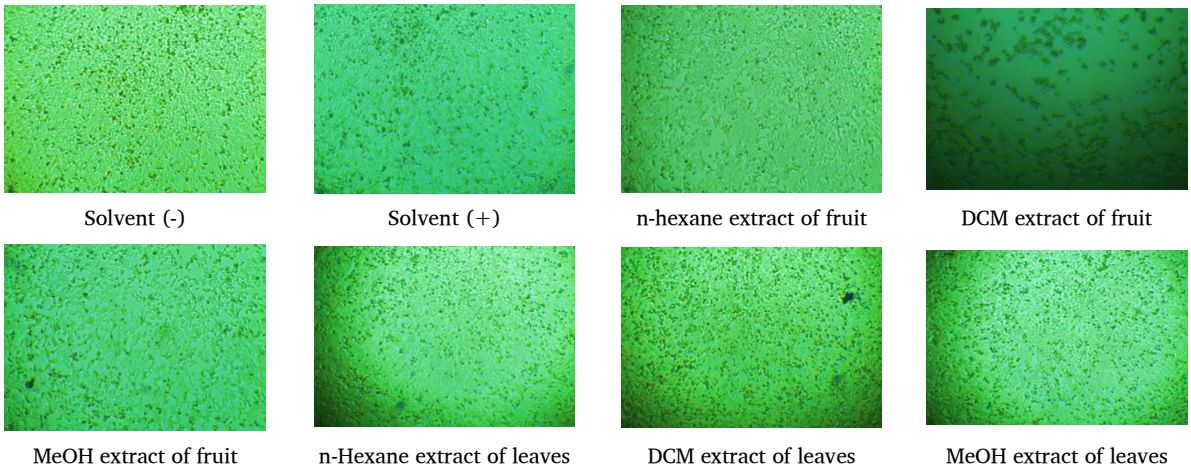


Fig. 6. Cytotoxicity of different extracts of *A. carambola* on HeLa cell line

4. Conclusion

The phytochemical screening of the extracts of *A. carambola* revealed the presence of lignin, quinones, saponins, flavonoids, phenol, carbohydrates, steroids, and terpenoids. The DCM extract of *A. carambola* leaves was investigated through chromatographic and spectroscopic techniques. Three biologically active compounds, β-sitosterol, 6,9-dieneoctadeca-1-ol, and palmitic acid, were identified in the DCM extract of *A. carambola*. The n-hexane extract of fruits mainly contained palmitoleic acid (20.94%) and octadecanoic acid (48.48%), while in leaves, it mainly contained palmitoleic acid (6.47%), octadecanoic acid (23.23%), and behenic acid (41.70%). DCM and MeOH extracts exhibited higher DPPH free radical scavenging activity than the n-hexane extract. The antibacterial screening showed no activity against *B. cereus* and *E. coli* bacteria. The antifungal activity of the different solvent extracts was determined using the well diffusion method against *Candida sp.* and *A. niger* fungi, but no activity was observed. In terms of cytotoxicity, the DCM extract of fruits showed cytotoxicity against the Vero cell line.

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Conflict of interest

The authors declare there is no conflict of interest in this study.

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