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PRELIMINARY PHYTOCHEMICAL SCREENING AND BIOACTIVITY INVESTIGATIONS OF AVORRHEA CARAMBOLA L.

Mifta Hul Jannah[1], Abdullah Al Shamsh Prottay[2], Mohammed Mashrur Chowdhury[1], Md. Bayejid Ahmed[3], M Mohi Uddin Chowdhury[1], and Muhammad Torequl Islam[2]*

[1]Department of Pharmacy, Southern University Bangladesh, Chattagram 4210, Bangladesh
[2]Department of Pharmacy, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj 8100, Bangladesh
[3]Department of Pharmacy, Jagannath University, Dhaka 1100, Bangladesh

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Abstract

Averrhoa carambola L. (*A. carambola*) is particularly well-known for its distinctive star-shaped fruit (Star fruit), cultivated in Southeast Asia, the Indian subcontinent, and northern and southern America. Historically, it has been used for a long time to cure many ailments, including vomiting, fever, arthralgia, cough, hypertension, hangovers, chronic paroxysmal headache, hyperglycemia, and diabetic nephropathy. This study aimed to examine its preliminary phytochemical and some biological properties. For this, fresh leaves of *A. carambola* were extracted (hot and cold) with ethanol, which was then fractionated with n-hexane and chloroform. Following a preliminary investigation of phytochemicals, radical scavenging, membrane stabilizing, alpha-glucosidase inhibitory, and clot lysis effects were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, egg albumin model, alpha-amylase assay, and human blood clot lysis assay, respectively using suitable reference standards. The results suggest that *A. carambola* contains alkaloids, glycosides, steroids, tannins, flavonoids, saponins, and reducing sugars. All the organic fractions demonstrated significant ($p < 0.05$) radical scavenging, membrane stabilizing, alpha-glucosidase inhibitory, and clot lysis activities in a concentration-dependent approach. Except for DPPH radical scavenging, the ethanol fraction showed better activities than the n-hexane and chloroform fractions. In summary, *A. carambola* might be a potential source of antioxidant, anti-inflammatory, hypoglycemic, and atherothrombolytic agents. Further studies are required to isolate, characterize, and establish molecular mechanisms for each component's biological effects.

Keywords: *Avorrhea carambola*, free radical scavenging, membrane stabilizing, glucosidase inhibition, clot lysis

Introduction

Since the beginning of civilization, people have used medicinal herbs. According to the World Health Organization (WHO), more than 80% of the earth's population uses natural remedies to manage their health issues, especially the millions who reside in large, isolated areas of developing countries (Mushtaq et al., 2018). Exogenous and indigenous treatments, as well as medicinal plants used to cure a variety of ailments, provide major economic advantages. The finest sources of bioactive molecules may be found in medicinal plants, shrubs, and trees, which are also essential components of the current global healthcare system (Asfaw et al., 2022). Each era experiences an increase in the utilization of plant-based medications, or phytomedicine, since they are safe, have few adverse effects, and work synergistically with other chemicals (Najmi et al., 2022). Several unique substances have been found and synthesized/isolated in contemporary facilities based on conventional techniques, philosophies, beliefs, and recorded or observed treatments across time (Wangkheirakpam, 2018). Many possible bioactive chemicals found in medicinal plants and a variety of foods have been shown to have important health benefits, for examples curcumin, quercetin, rosmarinic acid etc. (Mandal et al., 2022). Additionally, there is growing proof that bioactive substances have the ability to control different gene expressions and treat a number of disease situations, including oxidative stress, inflammation,

*Corresponding author: <dmt.islam@bsmrstu.edu.bd>
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cancer, hypertension, diabetes, and other diseases and disorders (Ortega & Campos, 2019). Various biological actions have been performed against them in both *in-vitro* and *in-vivo* studies (Sharifi-Rad et al., 2021).

Averrhoa carambola L. (*A. carambola*), an evergreen plant of the Oxalidaceae family, is commonly named "Kamranga" in Bangladesh. It is extensively produced in Southeast Asia and the Indian Subcontinent and is also referred to as "star fruit" (Islam et al., 2020). Additionally, it is a well-liked fruit in the markets of the South Pacific Islands, Australia, and the United States (Muthu et al., 2016). *A. carambola* is a kind of woody plant with a moderate growth rate. It has several stems and a small trunk, and it may reach optimum heights of 6 to 9 m. It has a bushy look with numerous branches that form a wide, spherical crown and a trunk base that can be up to 15 cm in diameter (Kapoor, 2018). Aside from having leaflets that fold together at dusk, the tree also exhibits sensitivity to light and shock through the sudden movements of its leaves. Small clusters of five-petaled red, lilac, or purple blooms are produced by the starfruit's shrub. Unripe fruits are often tiny and dark green; while, fully ripe fruits have a golden color. Typically, starfruits have five longitudinal ridges or angles, are juicy, and have a crunchy, crisp quality. Additionally, the fruit is known as "star fruit" because it has a star shape when cut horizontally (Muthu et al., 2016).

Herbal remedies are currently gaining popularity as an alternative to pharmacological therapy on a global scale (Lippert & Renner, 2022). Star fruits are used as a food source as well as an herb in various regions of Brazil, China, India, Malaysia, and Taiwan (Patel et al., 2015). In addition, it is also used in Ayurvedic and Traditional Chinese Medicine systems for the treatment of fever, cough, respiratory diseases, chronic paroxysmal headache, and epidermal inflammations (Wang et al., 2016). Various plant parts, including fruits, leaves, and stems, have been revealed to have pharmacological properties. According to phytochemical and pharmacological research, the extracts of the star fruit plant's leaves, fruits, and roots may include saponins, steroids, flavonoids, glycosides, alkaloids, carbohydrates, and tannins, all of which are reported to have antioxidant and particular therapeutic effects (Annegowda et al., 2012; Saikia et al., 2015). It has been demonstrated that the plant has biological properties that are anti-inflammatory, antioxidant, antimicrobial, and anti-ulcer (Dasgupta et al., 2013). Additionally, the high fiber content of fruits helps to absorb glucose while delaying its absorption into the bloodstream, which helps to regulate blood glucose levels (Wu et al., 2009). As it facilitates the elimination of cholesterol, lipids, and bile acid through excretion, starfruit consumption also has a hypocholesterolemic and hypolipidemic effect (Ferreira et al., 2008).

This study aimed at a preliminary phytochemical investigation along with the screening of free radical scavenging, membrane stabilizing, alpha-glucosidase inhibitory, and clot lysis effects of *A. carambola*'s organic extracts.

Materials and Methods

Collection, identification, grinding, and extraction of plant materials

Throughout June and July, fresh leaves were collected from the Sitakunda Eco Park's hill tracts in Chattagram. The leaves were then cleansed with running tap water. After that, the plant components were dried under the shade (at a temperature not going beyond 40 °C). The plant was recognized by the taxonomist at the Bangladesh Forest Research Institute Herbarium (BFRIH), Chattagram. A voucher sample was submitted as SA (525). Thereafter, the dried plant components were crushed into a coarse powder and maintained in an airtight container in a dry, cold, and dark location until the extraction process started.

In an amber-colored glass bottle, 800 ml of absolute ethanol was added to 200 g of coarse powder. It was closed and occasionally agitated. The mixture was filtered after 20 days using Whatmann filter paper no 1 after first passing through a cotton plug. The Soxhlet device performed a hot extraction on the marc that was recovered from the maceration process. Briefly, in a Soxhlet device (Quickfit, England), 1000 ml of absolute ethanol and 140 g of marc were combined. The resulting extract was collected, filtered, and subjected to a process that caused the solvent to evaporate below 60 °C.

Fractionation of crude extract

The procedure developed by Abubakar and Haque (2002) was used for solvent-solvent partitioning. Briefly, the crude ethanol extract (EAC: 18 g) was diluted in double-distilled water (DDW) and afterward fractionated using a fractionating column with n-hexane (HAC), and finally with chloroform (CAC). Until 150 ml of n-hexane and chloroform were produced, fractionation was performed using 50 ml of solvent each time, and after each fractionation, the mixture was vigorously shaken before being left to stand. Layers of solvent were divided and decanted. The leftover

extract was used as an ethanol fraction (EAC). The extracted materials were collected, filtered, and driven to have the solvent evaporate at a temperature below 50 °C. A sticky concentration was produced as a result of solvent evaporation. The sticky extracts were weighed and preserved in an airtight, clean, appropriately labeled container at 4 °C. Figure 1 shows the fractionation procedures of EAC of *A. carambola*.

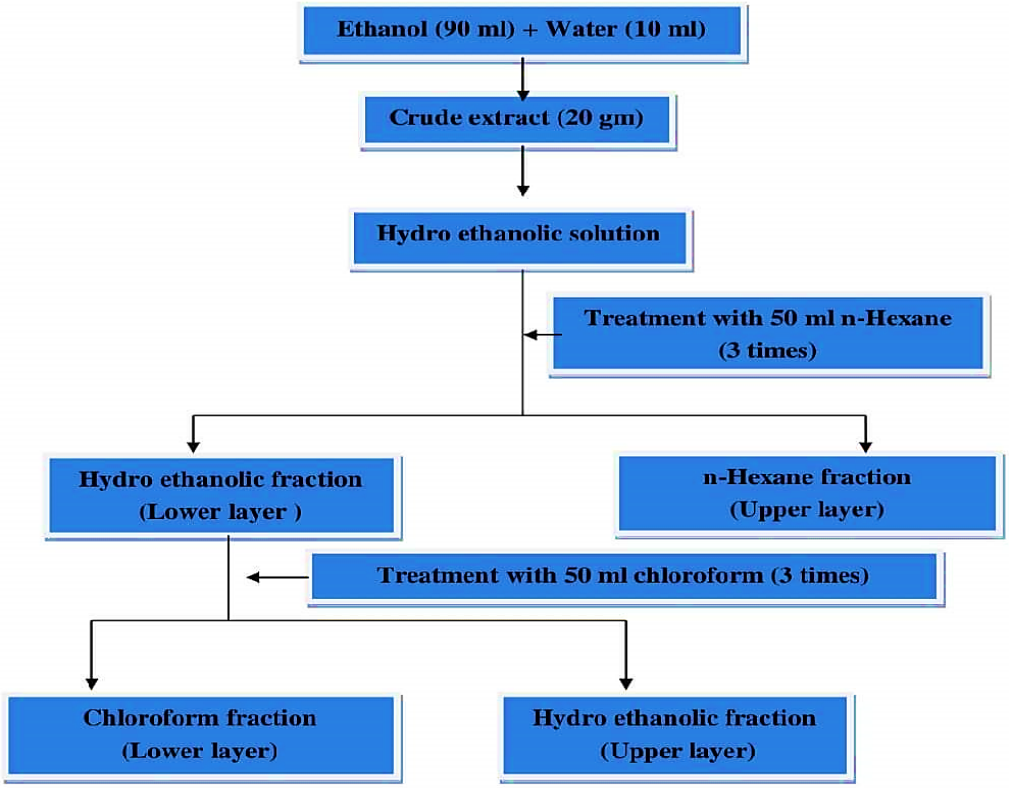

Figure 1. Schematic presentation of fractionation process of crude ethanol extract of *Averrhoa carambola*

Reagents and chemicals

Ethanol, n-hexane, chloroform, ascorbic acid, alpha-amylase, and DPPH were purchased from Merck India, while diclofenac sodium and acarbose were kindly provided by Square Pharmaceuticals Ltd. and Pacific Pharmaceuticals Ltd., both in Bangladesh. Streptokinase (Durakinase Injection; powder for reconstitution; 30,000 units per vial) was imported from Dong Kook, Korea.

Phytochemical screening

Preliminary phytochemical studies were done according to the method described by Islam (2021).

Free radical scavenging test (DPPH assay)

With a few minor adjustments, this investigation was carried out using the methodology outlined by Valko et al. (2007). Briefly, 0.5% tween-80 dissolved in normal saline was used as a vehicle to prepare all the fractions and the standard medication ascorbic acid (AA) at 20, 40, 60, 80, and 100 µg/ml. In methanol, a 0.24% DPPH solution was prepared. 100 µl of test or control solution are included in 3 ml of adequately mixed DPPH solution, which is then kept in dark for 30 minutes. A spectrometer (Shimadzu, Japan) was used to measure the absorbance at 517 nm, and the

following equation was used to calculate the percentage of the radical scavenging capability of the test or control samples:

$$\%\text{Scavenge of free DPPH radicals} = \frac{(Ac - At/s)}{Ac} \times 100$$

Where, Ac and At/s mean absorbance of control and test or standard, respectively.

The IC_{50} (the concentration needed to suppress 50% of the enzymatic activity) was estimated using a regression equation derived by plotting concentrations in the range of 20–100 µg/ml (x-axis) and inhibition (y-axis) for several treatments.

Membrane stabilization test (Egg albumin assay)

This experiment was carried out with a few minor adjustments to the procedure outlined by Mehta et al. (2017). In a summary, a 5 ml reaction mixture contained 2 ml of test or control samples, 2.8 ml of phosphate buffer (PBS, pH 6.4), and 0.2 ml of egg albumin. The standard (diclofenac sodium, DCLO) and test (EAC, CAC, and HAC) were examined at the same concentration as described in the preceding research. For this aim, the identical vehicle was also utilized as a control. The reaction mixtures were then incubated for a further 15 min at 37.2 °C and a further min at 70 °C. After that, a colorimeter was used to quantify the optical density at 660 nm. The following formula was used to calculate the percentage inhibition of protein denaturation:

$$\%\text{Inhibition of egg albumin denaturation} = 100 \times \frac{1 - (OD_2 - OD_1)}{(OD_3 - OD_1)}$$

Where, OD_1, OD_2, and OD_3 mean the optical densities of test (unheated), test (heated), and control (heated), respectively.

The IC_{50} (the concentration needed to suppress 50% of the enzymatic activity) was estimated using a regression equation derived by plotting concentrations in the range of 20–100 µg/ml (X-axis) and inhibition (Y-axis) for several treatments.

Glucosidase inhibitory test (Alpha-amylase assay)

With a few minor adjustments, this analysis was carried out in accordance with Pistia-Brueggeman and Hollingsworth (2001). In a brief, 50 µl of fractions or standards, such as acarbose (ACB) at different concentrations (20-100 g/ml), were incubated for 20 min at 37 °C with 10 µl of the -glucosidase (maltase) enzyme solution, along with 125 µl of 0.1 M PBS (pH 6.8). The addition of 20 µl of 1 M pNPG (substrate) to the reaction was made after 20 min had elapsed, and the mixture was then incubated for 30 min. A colorimeter was used to determine the final optical density at 405 nm after the reaction was stopped by the addition of 0.1 N of Na2CO3 (50 µl). The vehicle described before operated as an NC. Enzyme activity was analyzed as follows:

$$\%\text{Inhibition of alpha-amylase} = \frac{OD_B - OD_{T/S}}{OD_B} \times 100$$

Where, OD_B and $OD_{T/S}$ mean the optical densities of blank and test/standard, respectively.

The IC_{50} (the concentration needed to suppress 50% of the enzymatic activity) was estimated using a regression equation derived by plotting concentrations in the range of 20–100 µg/ml (x-axis) and inhibition (y-axis) for several treatments.

Anti-atherothrombosis test (Clot lysis assay)

This project using human sample was approved by the Ethical Committee (SUB/IAEC/12.01). With a few minor changes, this experiment was carried out by the procedure outlined by Hussain et al. (2014). In a brief, 10 healthy participants of either sex had their venous blood collected, and the blood was then promptly distributed to pre-

weighed, sterile micro-centrifuge tubes (500 µl blood/tube, 10 tubes for test or control). The tubes received 200 µl of 2% calcium chloride, were thoroughly mixed, and incubated for 45 min at 37°C to promote clotting. The serum was entirely withdrawn after that (without disrupting the clot), and the clot's weight was determined (weight of the clot-containing tube minus the weight of the tube alone). Marked tubes received 500 µl of the test or control solution, which was then incubated again at 37 °C for 90 min. The tubes labeled "control" were treated with the aforementioned vehicle and 30,000 IU of streptokinase (STK). Following the incubation period, the fluids that had been lysed were meticulously collected and weighed once again to calculate the percentage of clot lysis, as shown below:

%Clot lysis = (Weight of the clot after lysis by test or controls and removal of serum ÷ Weight of the clot before lysis by test or controls) × 100

Statistical analysis

The values are mean ± SD (standard deviation). One-way analysis of variance (ANOVA) followed by the *t*-student test. GraphPad Prism (version 6.5) is used to analyze the data. Values are considered significant when $p < 0.05$ at 95% confidence intervals.

Results

Extraction and fractions

Cold and hot extraction processes resulted in yields of 10 and 8 g (total 18 g), respectively. The percentage yield values for EAC, CAC, and HAC were 2.132, 2.05, and 3.276 g, respectively.

Phytochemical group test

The EAC contains glycosides, alkaloids, tannins, saponins, and flavonoids, whereas the CAC contains glycosides, tannins, and flavonoids. On the other hand, HAC possesses glycosides, tannins, flavonoids, saponins, and reducing sugars. There are no gums or amides in any of the fractions.

Table 1. Phytochemical groups observed in different fractions of *Averrhoa carambola*

Extracts	Phytochemical groups						
	Alkaloids	Glycosides	Steroids	Tannins	Flavonoids	Saponins	Reducing sugars
EAC	-	+	-	++	+++	+	+
CAC	+	+++	-	+	+	-	-
HAC	-	++	-	+	+	+	+

'+' = present, '-'= absent; multiplication of plus (+) sign means observed intensity of observation; EAC: ethanol extract of Averrhoa carambola; CAC: chloroform extract of Averrhoa carambola; HAC: n-hexane extract of Averrhoa carambola

Free radical scavenging test (DPPH assay)

The control (NC) exhibited negligible DPPH scavenging capacity (1.23±0.86). All the fractions exhibited significant ($p < 0.05$) concentration-dependent radical scavenging effects on DPPH radicals, where at 100 µg/ml EAC, CAC, and HAC showed percent inhibition of 40.42±2.91, 63.97±1.22, and 59.35±2.13%, respectively. AA at the same concentration also exhibited a significant ($p < 0.05$) percent inhibition of 83.37±1.09%. The IC_{50} determined for EAC, CAC, HAC, and AA were 48.01±1.23, 62.29±2.13, 78.92±1.67, and 21.53±2.13 µg/ml, respectively. CAC exhibited a better DPPH radical scavenging effect than the other fractions. However, the standard antioxidant AA exhibited a better radical scavenging effect than all fractions of *A. carambola* (Table 2).

Table 2. DPPH radical scavenging capacity of different fractions of *Averrhoa carambola* and controls

µg/ml	Percentage DPPH radical scavenge				
	NC	EAC	CAC	HAC	AA
20	1.23±0.86	40.42±1.08*	33.03±1.19*	19.86±1.78*	49.42±1.23*
40		47.34±2.18*	43.42±0.58*	28.41±1.53*	56.58±1.17*
60		58.19±0.78*	49.88±1.87*	42.49±2.13*	68.59±2.13*
80		67.21±1.13*	55.66±1.78*	53.35±2.09*	77.37±1.08*
100		40.42±2.91*	63.97±1.22*	59.35±2.13*	83.37±1.09*
IC_{50}	-	48.01±1.23	62.29±2.13	78.92±1.67	21.53±2.13

*Values are mean ± SD (standard deviation) (n = 5); *p <0.05 when compared to the NC group; DPPH: 2,2-diphenyl-1-picrylhydrazyl; EAC: ethanol extract of Averrhoa carambola; CAC: chloroform extract of Averrhoa carambola; HAC: n-hexane extract of Averrhoa carambola; NC: vehicle (0.5% tween-80 dissolved in normal saline); AA: ascorbic acid; IC_{50}: half maximal inhibitory concentration*

Membrane stabilization test (Egg albumin assay)

The NC produced negligible membrane stabilizing capacity (1.39±0.78%). All the fractions exhibited significant (p <0.05) concentration-dependent inhibitory effects on egg protein denaturation, where at 100 µg/ml EAC, CAC, and HAC showed percent inhibition of 76.91±1.09, 65.72±1.22, and 62.44±2.13%, respectively. DCLO at the same concentration also exposed a significant (p <0.05) percent inhibition of 89.22±2.91%. The IC_{50} determined EAC, CAC, HAC, and DCLO were 39.63±1.20, 76.78±2.13, 79.62±1.67, and 30.05±1.03 µg/ml, respectively. EAC revealed a better inhibitory result than the other fractions. However, the standard drug DCLO showed a better inhibitory outcome than all fractions of *A. carambola* (Table 3).

Table 3. Membrane stabilization capacity of different fractions of *Averrhoa carambola* and controls

µg/ml	Percentage inhibition of protein denaturation				
	NC	EAC	CAC	HAC	DCLO
20		37.67±1.23*	32.26±1.19*	21.22±1.78*	42.26±1.08*
40		50.62±1.17*	40.97±0.58*	31.29±1.53*	55.35±2.18*
60	1.39±0.78	58.29±2.13*	48.67±1.87*	42.59±2.13*	63.22±0.78*
80		70.19±1.08*	57.67±1.78*	51.10±2.09*	76.13±1.13*
100		76.91±1.09*	65.72±1.22*	62.44±2.13*	89.22±2.91*
IC_{50}	-	39.63±1.20	76.78±2.13	79.62±1.67	30.05±1.03

*Values are mean ± SD (standard deviation) (n = 5); *p <0.05 when compared to the NC group; EAC: ethanol extract of Averrhoa carambola; CAC: chloroform extract of Averrhoa carambola; HAC: n-hexane extract of Averrhoa carambola; NC: vehicle (0.5% tween-80 dissolved in normal saline); DCLO: diclofenac sodium; IC_{50}: half maximal inhibitory concentration*

Table 4. Alpha-amylase inhibition capacity of different fractions of *Averrhoa carambola* and controls

µg/ml	Percentage alpha-amylase activity inhibition				
	NC	EAC	CAC	HAC	ACB
20	1.56±0.53	39.61±1.23*	33.07±1.19*	7.88±1.78*	42.96±1.08*
40		49.35±1.17*	41.07±0.58*	20.30±1.53*	53.27±2.18*
60		58.44±2.13*	51.73±1.87*	30.00±2.13*	62.89±0.78*
80		65.91±1.08*	60.80±1.78*	40.61±2.09*	78.01±1.13*
100		79.55±1.09*	70.93±1.22*	51.82±2.13*	94.85±2.91*
IC_{50}	-	44.77±1.20	57.59±2.13*	99.19±1.67	27.56±1.03

Values are mean ± SD (standard deviation) (n = 5); EAC: ethanol extract of Averrhoa carambola; CAC: chloroform extract of Averrhoa carambola; HAC: n-hexane extract of Averrhoa carambola; NC: vehicle (0.5% tween-80 dissolved in normal saline); ACB: acarbose; IC_{50}: half maximal inhibitory concentration

Glucosidase inhibitory test (Alpha-amylase assay)

The NC produced negligible glucosidase inhibitory capacity (1.56±0.53%). All the fractions exhibited significant ($p < 0.05$) concentration-dependent inhibitory effects on the alpha-amylase enzyme, where at 100 µg/ml EAC, CAC, and HAC showed percent inhibition of 79.55±1.09, 70.93±1.22, and 51.82±2.13%, respectively. ACB at the same concentration (100 µg/ml) also presented significant ($p < 0.05$) percent inhibition of 94.85±2.91%. The IC_{50} determined EAC, CAC, HAC, and ACB were 44.77±1.20, 57.59±2.13, 99.19±1.67, and 27.56±1.03 µg/ml, respectively. EAC displayed a better inhibitory activity than the other fractions. However, the standard drug ACB revealed a better inhibitory property than all fractions of *A. carambola* (Table 4).

Anti-atherothrombosis test (Clot lysis assay)

Table 5 suggests that the control (NC) group exhibited negligible (1.94±0.79%) clot lysis capacity. The standard drug STK showed a significant ($p < 0.05$) and highest clot lysis capacity of 81.54±1.11%. Among the organic fractions, EAC exhibited better clot lysis capacity (19.93±0.86%), then followed by CAC (12.78±0.46%) and HAC (7.05±0.35%). However, the activity is poor when compared to the STK.

Table 5. Clot lysis capacity of different fractions of *Averrhoa carambola* and controls

Treatments	Percentage clot lysis
NC (vehicle)	1.94±0.79
STK	81.54±1.11*
EAC	19.93±0.86*
CAC	12.78±0.46*
HAC	7.05±0.35*

*Values are mean ± SD (standard deviation) (n = 10); *p <0.05 when compared to the NC group; EAC: ethanol extract of Averrhoa carambola; CAC: chloroform extract of Averrhoa carambola; HAC: n-hexane extract of Averrhoa carambola; NC: vehicle (0.5% tween-80 dissolved in normal saline); STK: streptokinase*

Discussion

Ethanol, chloroform, and n-hexane are frequently used for the extraction of compounds present in plants. Hydrophilic compounds are extracted with ethanol, methanol, or ethyl acetate, while lipophilic compounds are extracted using chloroform, dichloromethane, or a mixture of dichloromethane/methanol. On the other hand, n-hexane is used to remove chlorophyll from plant extracts (Cosa et al., 2006). For initial extraction, chloroform is used for dried plant materials. However, this solvent is frequently used to extract glycosidal contents from the crude samples (Onwukaeme et al., 2007). To date, approximately 132 phytoconstituents have been isolated from *A. carambola*. Among them, glycosides, flavonoids, and benzoquinones are evidently bioactive (Luan et al., 2021). Our preliminary phytochemical report suggests that EAC showed high intensities for tannins and flavonoids, while CAC and HAC showed high intensities for glycosides.

The DPPH test is a straightforward and accurate way to assess the capacity of a stable free radical called DPPH (2,2-diphenyl-1-picrylhydrazyl) to decolorize when antioxidants are present (Bao et al., 2020). It is a rapid and efficient colorimetric technique that works by reducing DPPH radicals in the presence of a proton donor material, which produces diamagnetic molecules (Diniso et al., 2022). For this test, AA was used as the standard antioxidant. The absorbance at 515 to 517 nm as well as the apparent deep purple color are caused by the odd electron that the DPPH radical possesses. The amount of decolorization caused by DPPH accepting an electron given to it by an antioxidant chemical may be calculated from changes in absorbance (Hasan et al., 2009). Phenolic chemicals such as polyphenols, tannins, flavonoids, and phenolic terpenes have a significant influence on the antioxidant property of plant products (Nguyen et al., 2020). The major source of phenolic chemicals' antioxidant action is their redox characteristics, which can be useful for adsorbing and neutralizing free radicals, reducing singlet and triplet oxygen, or dissolving peroxides (Laouicha et al., 2020). Moreover, research evidence suggests that the plant contains high levels of vitamin C (25.8 mg/100 g fruit) (Muthu et al., 2016). One study reports that *A. carambola extract at* 0.1–375 µg/ml showed significant DPPH radical scavenging capacity, where IC_{50} was calculated at 55.55 to 100.0 µg/ml (Liao et al., 2019; Islam et al.,

2020; Siddika et al., 2020). In this study, we have seen that all the fractions exhibited DPPH radical scavenging capacity within the IC_{50} values 48.01±1.23 and 78.92±1.67 µg/ml, suggesting agreement with the previous reports. A variety of human neurological and other diseases, including inflammatory responses, viral infections, immunological abnormalities, and digestive tract disorders like GI inflammation and ulceration, currently seem to be caused by oxidative stress as their core mechanism (Aruoma, 2003). Comparable to this, in the complex phases of carcinogenesis, reactive oxygen species (ROS) begin with DNA damage and the accumulation of genetic alterations through one or multiple cell lines, which progress to dysplastic cellular appearance, uncontrolled cell proliferation, and ultimately cancer (Saretzki, 2010). Therefore, treatment with antioxidants that scavenge free radicals can prevent, postpone, or improve many of these diseases (Akbari et al., 2022). A growing amount of data from epidemiological and clinical investigations over the last two decades has shown that several plants or their recognized antioxidant-rich components have significant inhibitory impacts on human carcinogenesis (Hasan et al., 2009). According to the current findings, *A. carambola* may play a part in the biological action of antioxidants. DPPH radicals were significantly scavenged by the EAC, CAC, and HAC as compared to the AA reference standard at a concentration of 100 µg/ml. Compared to the other fractions, CAC showed a stronger ability to scavenge DPPH radicals. The results of the experiment show that plant extracts have modest antioxidant action. Studies demonstrate that glycosides are promising natural antioxidants (Kumar et al., 2010; Wen et al., 2017). Our phytochemical reports suggest that CAC showed stronger intensities for glycosides.

The *in-vitro* anti-denaturation technique of egg albumin was adopted to assess the anti-inflammatory properties of *Averrhoa carambola*. The egg albumin is heated to a high temperature during the anti-denaturation technique test. Denatured proteins can express certain antigens. These antigens are linked to hypersensitivity responses (type-III), which are linked to a number of illnesses such as serum sickness and glomerulonephritis (Usman & Annamaraju, 2021). Proteins that have been heated up can cause delayed hypersensitivity. These proteins are just as effective as native proteins at stimulating cells (Yesmin et al., 2020). Furthermore, it has previously been established that traditional NSAIDs like diclofenac sodium reduce protein denaturation in addition to preventing the generation of endogenous prostaglandins by inhibiting the cyclooxygenase (COX) enzyme (Aidoo et al., 2021). Our results showed that EAC, HAC, and CAC, inhibited protein denaturation significantly as compared to the control group. The extracts at 100 µg/ml demonstrated appreciable anti-inflammatory action compared to the equivalent standard (DCLO) concentration. EAC revealed a better inhibitory result than the other fractions. Phytochemicals like alkaloids and flavonoids can give the plant its anti-inflammatory properties (Truong et al., 2019). Additional secondary metabolites produced by plants, such as terpenoids and glycosides, may potentially have anti-inflammatory activities (Shazhni et al., 2018). It is also evident that oxidative stress leading to inflammation or *vice versa* may lead to excessive tissue damage, which thereby results in numerous diseases and disorders in humans (Khansari et al., 2009). Therefore, the strong antioxidant capacity of *A. carambola* might be linked to its promising anti-inflammatory effect (in-vitro) in this present study.

A large number of medicinal herbs have been linked to antidiabetic action, which is regulated by a variety of mechanisms, including higher pancreatic insulin production, suppression of glucose absorption, and accelerated glucose uptake by muscles and adipose tissue (Elwekeel et al., 2022). The suppression of enzymes that metabolize carbohydrates, such as α-glucosidase and α-amylase, is another significant strategy recognized for the control of diabetes (Zhang et al., 2022). The conversion of oligosaccharides and disaccharides into monosaccharides, which may be transported through the intestinal mucosa and into the circulation, is carried out by these enzymes (Aggarwal et al., 2022). As a result of the suppression of α-glucosidase and α-amylase, blood glucose levels drop later than expected, which is followed by a reduction in insulin production (El-Manawaty & Gohar, 2018). In this investigation, the inhibitory impact of A. carambola extract against α-glucosidase enzyme was examined. When compared to standard ACB (IC50 = 94.85±2.91 µg/ml), the EAC (IC50 = 79.55±1.09 µg/ml) demonstrated strong inhibitory action against the α-amylase enzyme. A possible putative explanation for the A. carambola extract's strong hypoglycemia impact was identified by a literature review as having comparable α-amylase inhibitory actions (Chen et al., 2015; Hung et al., 2012). These findings imply that the inhibitory impact against enzymes that break down carbohydrates may be a potential mechanism for the anti-diabetic properties of A. carambola, suggesting that this plant's antidiabetic activity may include several targets.

One of the leading causes of morbidity and death around the globe is atherothrombosis, which is defined by the development of one or more atheromatous plaques within the blood vessels (Martin-Ventura et al., 2017). The clot-filled blood inside the arteries is lysed using fibrinolytic chemicals. Beta-hemolytic *Streptococci* bacteria release the extracellular fibrinolytic enzyme known as streptokinase, which is used frequently since it is inexpensive (Sikder et al., 2022). Herbal products may have anti-thrombotic properties, according to a body of research. It is clear that secondary metabolites originating from plants, such as polyphenols, alkaloids, and glycosides, have an anti-atherothrombotic effect (Olatunji et al., 2017; Zhu & Fang, 2014). In order to dissolve the clots in the blood vessels, it first forms a complex by connecting to both free and fibrin-bound plasminogen. After that, the complex converts the other free plasminogen into an active protease plasmin (Li et al., 2020). This medication is most frequently used to treat pulmonary embolism, deep vein thrombosis, and acute myocardial infarction (Dundar et al., 2003). Our research shows that *A. carambola* extracts have a considerable, concentration-dependent capability for clot lysis. According to research, there is potential for curing atherosclerosis by using medicinal herbs to lower cholesterolemia, free radicals, inflammatory diseases, and critical enzymes (Sedighi et al., 2017). Furthermore, the medicinal herb may be a great tool for treating individuals with hyperlipidemia and associated problems, either by itself or in conjunction with hypocholesterolemic medications (Wang et al., 2017).

Conclusion

This study reports that *A. carambola* possesses many important phytochemical groups, including alkaloids, glycosides, tannins, saponins, and flavonoids. It had significant DPPH radical scavenging, egg albumin stabilizing, and alpha-glucosidase inhibitory effects that were concentration dependent. However, it exhibited poor clot lysis capacity. We suppose that our observed bioactivities might be due to the presence of some important phytochemical groups, especially the glycosides and flavonoids in its fractions. The herb might be a potential source of antioxidant, anti-inflammatory, and anti-diabetic agents. Further research is required to isolate and characterize its bioactive components and elucidate the possible action mechanism for each of its bioactivities.

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Conflict of interest

The authors declare no conflict of interest.

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