



IN VITRO EVALUATION OF THE ANTI HYPERTENSIVE ACTIVITY OF AVERROHA CARAMBOLA EXTRACTS

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Abstract

Hypertension is a major cardiovascular disorder. It is characterized by a persistent elevation of blood pressure. Uncontrolled hypertension may damage the heart, kidneys, brain, and blood vessels. It is an important risk factor for cardiovascular disease and stroke. Current antihypertensive drugs are effective. However, long-term treatment may be associated with adverse effects. This has increased interest in natural products as potential sources of new therapeutic agents.

Averrhoa carambola L. belongs to the family Oxalidaceae. It is commonly known as star fruit. Different parts of the plant have been used in traditional medicine. The plant contains several phytoconstituents. These include phenolics, flavonoids, tannins, terpenoids, and other secondary metabolites. Such compounds may contribute to its biological activities.

The present study was designed to investigate the in-vitro antihypertensive potential of *A. carambola* extracts. The plant material was collected and authenticated. Suitable extraction procedures were used to obtain the plant extracts. Preliminary phytochemical screening was performed to identify major classes of constituents. The antihypertensive potential was assessed using an in-vitro enzyme inhibition assay. Angiotensin-converting enzyme inhibition was considered as an important screening parameter. Different extract concentrations were evaluated. The activity was compared with a suitable standard inhibitor.

The study provides preliminary information on the antihypertensive potential of *A. carambola*. The findings may help identify promising plant extracts for further investigation. Isolation of active constituents and detailed pharmacological studies may support future development of plant-based antihypertensive agents.

Keywords: *Averrhoa carambola*, hypertension, antihypertensive activity, ACE inhibition, phytochemicals, medicinal plant, plant extract.

1. INTRODUCTION

Hypertension

Hypertension is a chronic disorder of the cardiovascular system. It is characterized by persistently elevated arterial blood pressure. It is one of the most common non-communicable diseases worldwide. Many affected individuals remain unaware of their condition. This is because hypertension may not produce clear symptoms in its early stages [1,2].

Persistent high blood pressure can cause progressive vascular damage. It can affect the heart, brain, kidneys, and blood vessels. It increases the risk of coronary heart disease and stroke. It may also contribute to heart failure and chronic kidney disease [1,3].

Blood pressure is mainly determined by cardiac output and systemic vascular resistance.

$$\text{Blood Pressure} = \text{Cardiac Output} \times \text{Systemic Vascular Resistance}$$

Cardiac output depends on heart rate and stroke volume. Systemic vascular resistance depends mainly on vascular tone. An increase in either factor can increase blood pressure [2,4].

Several mechanisms contribute to hypertension. These include increased sympathetic activity, abnormal renal sodium handling, endothelial dysfunction, vascular stiffness, and activation of the renin–angiotensin–aldosterone system [2–4].

Types of Hypertension Primary Hypertension

Primary hypertension is also called essential hypertension. It does not have one specific underlying cause. It develops through several interacting factors. Genetic factors can contribute to its development. Age, obesity, high salt intake, physical inactivity, stress, and unhealthy dietary habits may also increase the risk [2,3].

Primary hypertension accounts for most cases in adults. The condition usually develops gradually. Multiple regulatory systems may be involved.

Secondary Hypertension

Secondary hypertension develops due to an identifiable cause. Kidney disorders are important causes. Endocrine abnormalities can also produce secondary hypertension. Certain medicines and substances may contribute to this condition [3,5].

Treatment of the underlying cause may improve blood pressure. Therefore, identification of secondary hypertension is clinically important.

Isolated Systolic Hypertension

Isolated systolic hypertension is characterized by increased systolic blood pressure with a relatively lower diastolic pressure. It is more common in older adults. Reduced arterial elasticity is an important contributing factor [2,3].

Resistant Hypertension

Resistant hypertension occurs when blood pressure remains above the desired level despite appropriate antihypertensive treatment. Several factors may contribute to this condition. These include poor adherence, obesity, secondary hypertension, and inappropriate treatment [3,6].

Pathophysiology of Hypertension

The pathophysiology of hypertension is complex. Several regulatory systems are involved. The kidneys, blood vessels, heart, brain, and endocrine system all contribute to blood pressure regulation.

Increased sympathetic nervous system activity can increase heart rate and vascular tone. This increases cardiac output and vascular resistance. Persistent sympathetic stimulation may therefore contribute to sustained hypertension [2,4].

The kidneys have an important role in long-term blood pressure control. Abnormal sodium excretion can increase extracellular fluid volume. This increases blood volume. Increased blood volume can increase cardiac output and arterial pressure [4].

Endothelial dysfunction is another important factor. The vascular endothelium produces substances that regulate vascular tone. Nitric oxide promotes vasodilation. Reduced nitric oxide availability can impair vascular relaxation. This may increase vascular resistance [7].

Vascular remodeling can also occur during prolonged hypertension. The walls of blood vessels may become thicker and less compliant. This can further increase vascular resistance. A vicious cycle may then develop [4,7].

Role of the Renin–Angiotensin–Aldosterone System

The renin–angiotensin–aldosterone system is an important regulator of blood pressure.

When renal perfusion decreases, the kidneys release renin. Renin acts on angiotensinogen. It produces angiotensin I. Angiotensin-converting enzyme then converts angiotensin I into angiotensin II [8].

Angiotensin II is a powerful vasoconstrictor. It increases vascular resistance. It also stimulates aldosterone secretion. Aldosterone increases sodium reabsorption in the kidneys. Water retention occurs along with sodium retention. This increases blood volume and contributes to elevated blood pressure [8,9].

Pathophysiological pathway

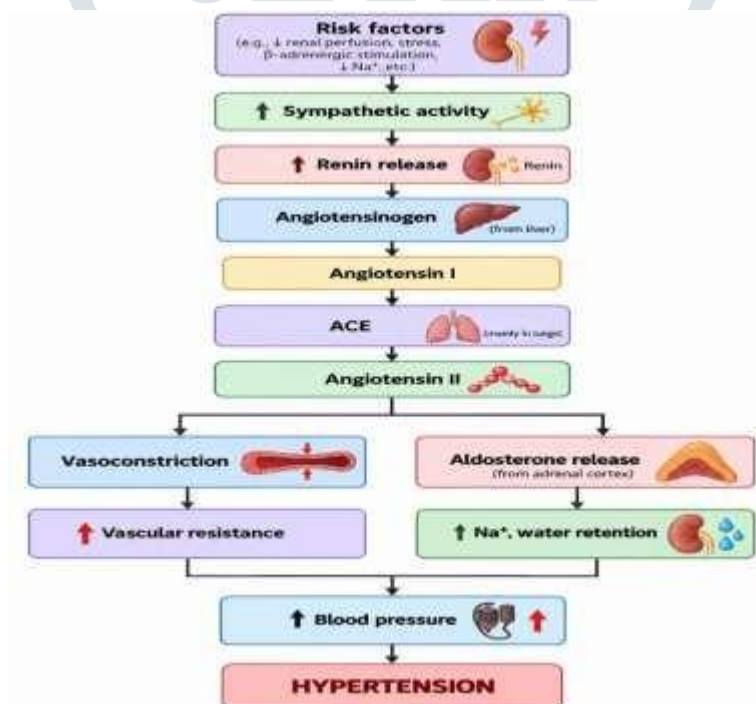


Figure:1 Pathophysiological pathway

ACE is therefore an important target for antihypertensive therapy. Inhibition of ACE decreases angiotensin II formation. It also increases the availability of bradykinin. These effects can promote vasodilation and reduce vascular resistance [8,9].

Introduction to *Averrhoa carambola*

Averrhoa carambola L. belongs to the family **Oxalidaceae**. It is commonly known as star fruit. The fruit has a characteristic five-ridged shape. When cut transversely, it resembles a star.

The plant is widely distributed in tropical and subtropical regions. It is cultivated for its edible fruit. Different parts of the plant have also been used in traditional medicine [12].



Figure2: *Averrhoa carambola*

The fruit and other plant parts contain several phytochemicals. Reported constituents include phenolic compounds, flavonoids, tannins, triterpenes, and other secondary metabolites [12,13].

The chemical composition of *A. carambola* may vary according to the plant part and extraction method. Solvent polarity can also influence the compounds obtained during extraction. Therefore, extracts prepared using different solvents may show different biological activities [13].

Phytochemical Basis of *A. carambola* Activity

Phenolic compounds are important constituents of many medicinal plants. They can interact with reactive oxygen species. They may therefore contribute to antioxidant activity.

Flavonoids are another important group. They have been investigated for antioxidant and cardiovascular effects. Some flavonoids may influence endothelial function and vascular signaling [14].

The presence of these compounds makes *A. carambola* an interesting candidate for pharmacological investigation. However, the presence of a phytochemical does not automatically establish a therapeutic effect. Experimental evaluation is required.

Mechanism of *Averrhoa carambola* Extract

For the present **in-vitro** study, ACE inhibition can be considered a major screening mechanism.

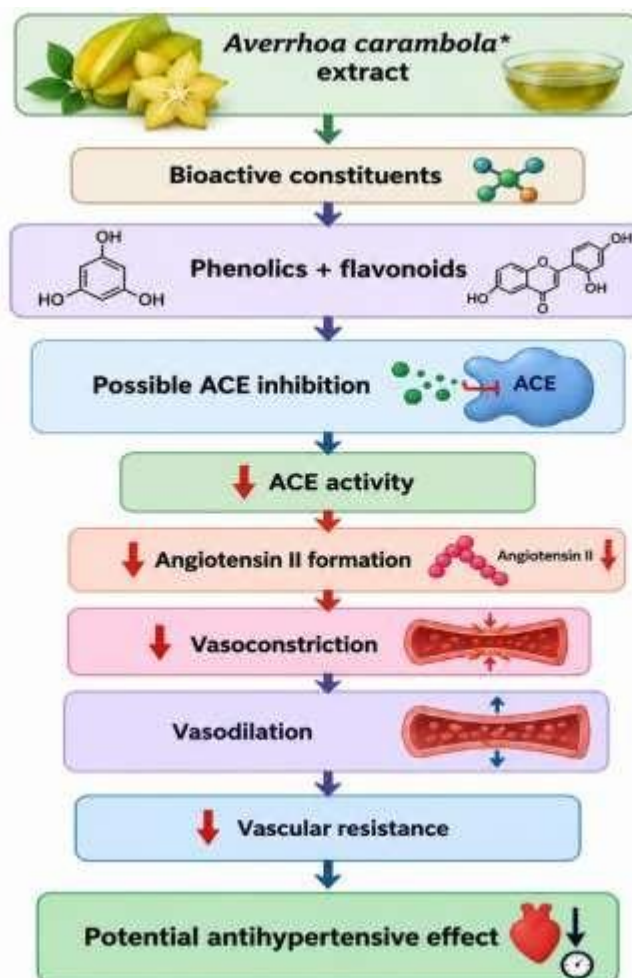


Figure3: Mechanism of *Averrhoa carambola* Extract

ACE Inhibition

ACE converts angiotensin I into angiotensin II. Angiotensin II promotes vasoconstriction. It also stimulates aldosterone release.

If compounds present in the extract inhibit ACE, angiotensin II formation may decrease. This can reduce vasoconstrictor activity. It may also reduce aldosterone-mediated sodium and water retention [8,9].

Therefore, ACE inhibition provides a rational target for evaluating the antihypertensive potential of *A. carambola* extracts.

2. AIM

To evaluate the in vitro antihypertensive potential of *Averrhoa carambola* fruit extracts by evaluating their ACE inhibitory and antioxidant activities.

OBJECTIVE

- To prepare extracts of *Averrhoa carambola* fruit using suitable solvents and standard extraction methods.
- To perform preliminary phytochemical screening of the prepared extracts to identify the major phytochemical constituents.
- To evaluate the in vitro ACE inhibitory activity of *Averrhoa carambola* extracts and determine their potential antihypertensive effects.
- To assess the antioxidant activity of the extracts using suitable in vitro antioxidant assays.
- To investigate the relationship between phytochemical constituents and the observed antihypertensive and antioxidant activities.

3. Material and Method-

The plant material selected for the present study was *Averrhoa carambola* Linn., belonging to the family Oxalidaceae. Fresh leaves and fruits were used for the preparation of plant extracts.

The plant is commonly known as carambola, kamrakh, and star fruit. These plant parts were selected for phytochemical screening and evaluation of their in vitro antihypertensive and antioxidant activities.



Figure 4: Averrhoa Carambola Fruit

3.1 Chemicals and Reagents

All the chemicals and reagents used in the study were of analytical grade.

Table1: Chemicals & Reagents

Sr. No.	Chemicals & Reagent
1.	Methanol
2.	Ethanol
3.	Distilled water
4.	Hydrochloric acid (HCl)
5.	Sodium hydroxide (NaOH)
6.	Potassium phosphate buffer
7.	Angiotensin-converting enzyme (ACE)
8.	Hippuryl-histidyl-leucine (HHL) (substrate for ACE assay)
9.	Standard drug (e.g., Captopril)
10.	Dragendorff's reagent (for alkaloids)
11.	Ferric chloride solution (for tannins)
12.	Mayer's reagent
13.	Lead acetate solution

All reagents were procured from Ishaanav Nutraceuticals Pvt Ltd., Selaqui, Dehradun and prepared according to standard laboratory procedures.



3.2 Collection and Authentication of Plant Material

Fresh, ripe fruits of *Averrhoa carambola* Linn. were collected from a local market or home garden in Sahaspur, Dehradun, Uttarakhand. The collected plant material was authenticated by Prof. Lalit Chauhan, a qualified botanist at JBIT Group of Institutions. A voucher specimen was prepared and preserved for future reference and verification.

3.3 PREPARATION OF PLANT EXTRACT

3.3.1 Collection and Preparation of Plant Material.

Fresh *Averrhoa Carambola* fruits and leaves were collected and thoroughly washed with distilled water to remove dirt and other contaminants. After that, they were left to dry in the shade at room temperature



Figure 5: Collection of Star fruit

Figure6: Washing of Star fruit

3.3.2. Shade Drying

The plant material was spread uniformly and it was dried under the shade at room temperature i.e., 20-25°C for about one week. Avoid direct sunlight to dry the plant material because it can degrade the heat sensitive and photo sensitive compounds of the plant. Ensure the end point of drying when the plant material became brittle and all the moisture was removed from them.



Figure7 : Shade Drying



Figure 8: Dried Star fruit

3.3.3 Shade Drying

The collected plant material was washed and cleaned properly. It was spread in a thin layer and dried under shade. The temperature was maintained at about 20–25°C. Drying was continued for about seven days. Direct sunlight was avoided. This helped to protect heat- and light-sensitive constituents. Drying was continued until the material became completely dry and brittle.

3.3.4 Size Reduction

The dried material was cut into small pieces. It was then powdered using a mechanical grinder. The powder was passed through **sieve no. 40**. This provided a uniform particle size. The powder was collected in a clean, dry, airtight container. It was stored until further extraction.



Figure 9 : Size Reduction by Chopping & Sieving

3.3.5 Extraction by Soxhlet Method

The powdered plant material was extracted using a Soxhlet apparatus. Ethanol and water were used as solvents.

Ethnolic Extraction

About 100 g of powdered material was placed in a Soxhlet thimble. About 500 mL of ethanol was used as the solvent. The solvent was heated in a round-bottom flask. The vapour condensed and passed through the plant material. This cycle was repeated continuously. Extraction was carried out for about seven hours. The process was stopped when the solvent became almost colourless.

Aqueous Extraction:

The residue obtained after ethanol extraction was dried. It was then extracted with water using the Soxhlet apparatus. The same extraction procedure was followed.

Concentration of Extracts:

The extracts were filtered through Whatman filter paper. The filtrates were concentrated using a water bath. The temperature was controlled during evaporation. A concentrated semi-solid extract was obtained. The extracts were transferred into airtight containers. They were stored at 4°C until further analysis.

3.3.6 Percentage Yield

The percentage yield of each extract was calculated using the following formula:

$$\text{Percentage yield (\%)} = (\text{Weight of dried extract} / \text{Weight of powdered material}) \times 100$$

The ethanolic extract showed a yield of 12.4%. The aqueous extract showed a yield of 8.6%. The total yield was 21.0%.

3.4 Preliminary Phytochemical Screening

The prepared extracts were subjected to preliminary phytochemical screening. The tests were used to detect major phytochemical groups. The extracts were tested for alkaloids, glycosides, flavonoids, saponins, tannins, phenolics, proteins, and carbohydrates.

3.4.1 Mayer's Test for Alkaloids

The extract was treated with Mayer's reagent. A cream-coloured precipitate indicated the presence of alkaloids.

3.4.2 Keller–Killiani Test for Glycosides

The extract was mixed with glacial acetic acid and ferric chloride. Concentrated sulphuric acid was added carefully. A brown ring at the junction indicated the presence of glycosides.

3.4.3 Shinoda Test for Flavonoids

The extract was treated with magnesium turnings and concentrated hydrochloric acid. A pink, red, or scarlet colour indicated the presence of flavonoids.

3.4.4 Foam Test for Saponins

The extract was diluted with distilled water. It was shaken vigorously. Persistent foam indicated the presence of saponins.

3.4.5 Test for Tannins

The extract was treated with ferric chloride solution. A dark blue or greenish-black colour indicated the presence of tannins.

3.4.6 Test for Phenolic Compounds

The extract was mixed with ferric chloride solution. Development of a dark colour indicated the presence of phenolic compounds.

3.4.7 Biuret Test for Proteins

The extract was treated with sodium hydroxide. Copper sulphate solution was then added. A violet colour indicated the presence of proteins.

3.4.8 Molisch's Test for Carbohydrates

The extract was treated with Molisch's reagent. Concentrated sulphuric acid was added carefully. A violet ring at the junction indicated the presence of carbohydrates.

3.5 ACE Inhibitory Assay – Principle

ACE converts angiotensin I into angiotensin II, which causes vasoconstriction and contributes to increased blood pressure. The ACE inhibitory assay measures the ability of a sample to reduce ACE activity.

In the present study, *Averrhoa carambola* extracts were added to the ACE reaction mixture. ACE inhibition reduced the formation of the reaction product. The change was measured using a spectrophotometric method. The percentage inhibition was calculated from the absorbance values.

Higher ACE inhibition indicates greater inhibition of ACE under the test conditions and suggests possible antihypertensive potential.

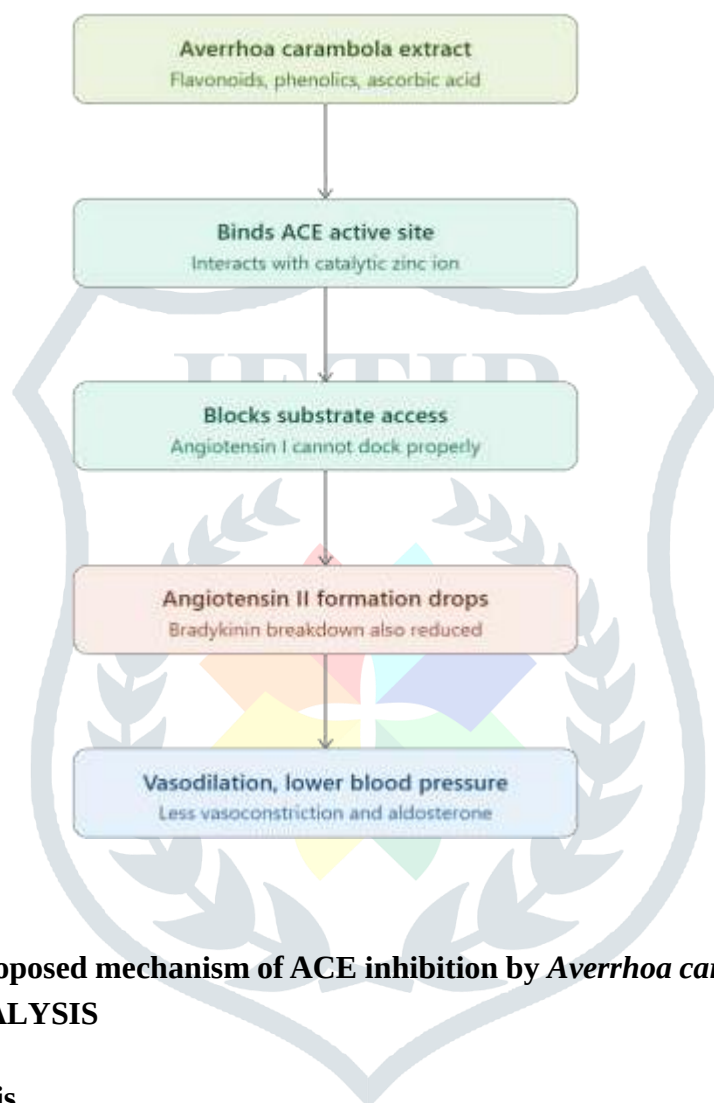


Figure 10: Proposed mechanism of ACE inhibition by *Averrhoa carambola* extract

3.6 STATISTICAL ANALYSIS

3.6.1 Statistical Analysis

All experimental measurements were performed in triplicate. The results were expressed as **mean ± standard deviation (SD)**. The ACE inhibitory activity of ethanolic and aqueous extracts was compared at different concentrations.

Microsoft Excel was used for data calculation and graphical presentation. The percentage ACE inhibition was calculated for each concentration.

One-way ANOVA was used to compare the different concentration groups. A **p-value < 0.05** was considered statistically significant.

The results showed an increase in ACE inhibition with increasing extract concentration. The ethanolic extract showed greater inhibition than the aqueous extract under the tested conditions.

3.6.2 Interpretation of Results

Both extracts showed concentration-dependent ACE inhibitory activity. The ethanolic extract produced greater

inhibition than the aqueous extract. The highest inhibition was observed at **400 µg/mL**.

The variation between the tested concentrations was statistically significant when **p < 0.05**. The findings indicate that *Averrhoa carambola* extracts have measurable in vitro ACE inhibitory activity.

The observed activity may be related to the phytochemicals present in the extracts, particularly flavonoids, phenolic compounds, tannins, alkaloids, and saponins. However, further studies are required to identify the specific compounds responsible for the activity.

The results provide preliminary support for further investigation of *Averrhoa carambola* as a potential source of ACE-inhibitory compounds.

4. RESULT:

4.1 Results of Extraction

The dried powdered of *Averrhoa Carambola* were extracted by Soxhlet extraction method using ethanol and aqueous extraction. The ethanolic extraction followed by aqueous extraction yielded a brown colour semi solid mass.

Table1: Result of Extraction

Extract	Weight of Powdered drug	Yield obtained	Percentage Yield
Ethanolic extract	100 g	12.4 g	12.4 %
Aqueous Extract	Residue	8.6 g	8.6 %
Total Yield		21.0 g	21.0 %

4.2 Phytochemical Screening

Phytochemical screening of *averrhoa carambola* extract was carried out for identification of various phytoconstituents. The result shows the presence of secondary metabolites like flavonoids, glycosides, alkaloids, tannin, etc.

Table 2: Result of Phytochemical Screening

Phytochemical constituents	Ethanolic Extraction	Aqueous Extraction
Alkaloids	Present (+)	Present (+)
Glycosides	Present (+)	Present (+)
Flavonoids	Strongly Present (++)	Present (+)
Saponins	Present (+)	Present (+)
Tannin	Present (+)	Present (+)
Phenolic Compound	Strongly Present (++)	Present (+)
Proteins	Present (+)	Present (+)
Carbohydrates	Present (+)	Present (+)

4.3 results of ACE Inhibitory Assay

The activity of Averrhoa Carambola extract as anti-hypertensive compound was evaluated by ACE Inhibitory Assay using different concentration.

A) Raw Observation Data (Ethanolic Extraction)

Table 3: Raw Data of Ethanolic Extraction

Concentration (µg/ml)	Absorbance	% Inhibition
50	0.712	23.0
100	0.598	39.0
200	0.441	58.0
400	0.289	76.0

Control Absorbance = 0.925

B) Raw Observation Table (Aqueous Extracts)

Table 4: Raw Data of Aqueous Extraction

Concentration (µg/ml)	Absorbance	% Inhibition
50	0.756	18.0
100	0.664	31.0
200	0.531	46.0
400	0.382	63.0

Control Absorbance = 0.925

C) ACE Inhibition Activity

Table 5: ACE Inhibition Activity

Concentration (µg/ml)	% Inhibition (Mean ± SD)	
	Ethanolic Extract	Aqueous Extract
50	23 ± 1.1	18 ± 1.0
100	39 ± 1.4	31 ± 1.2

200	58 ± 1.7	46 ± 1.5
400	76 ± 2.0	63 ± 1.8

D) Standard Drug Comparison

Table 6: Standard Drug Activity

Standard Drug	Concentration ($\mu\text{g/ml}$)	% Inhibition
Captopril	100	88%

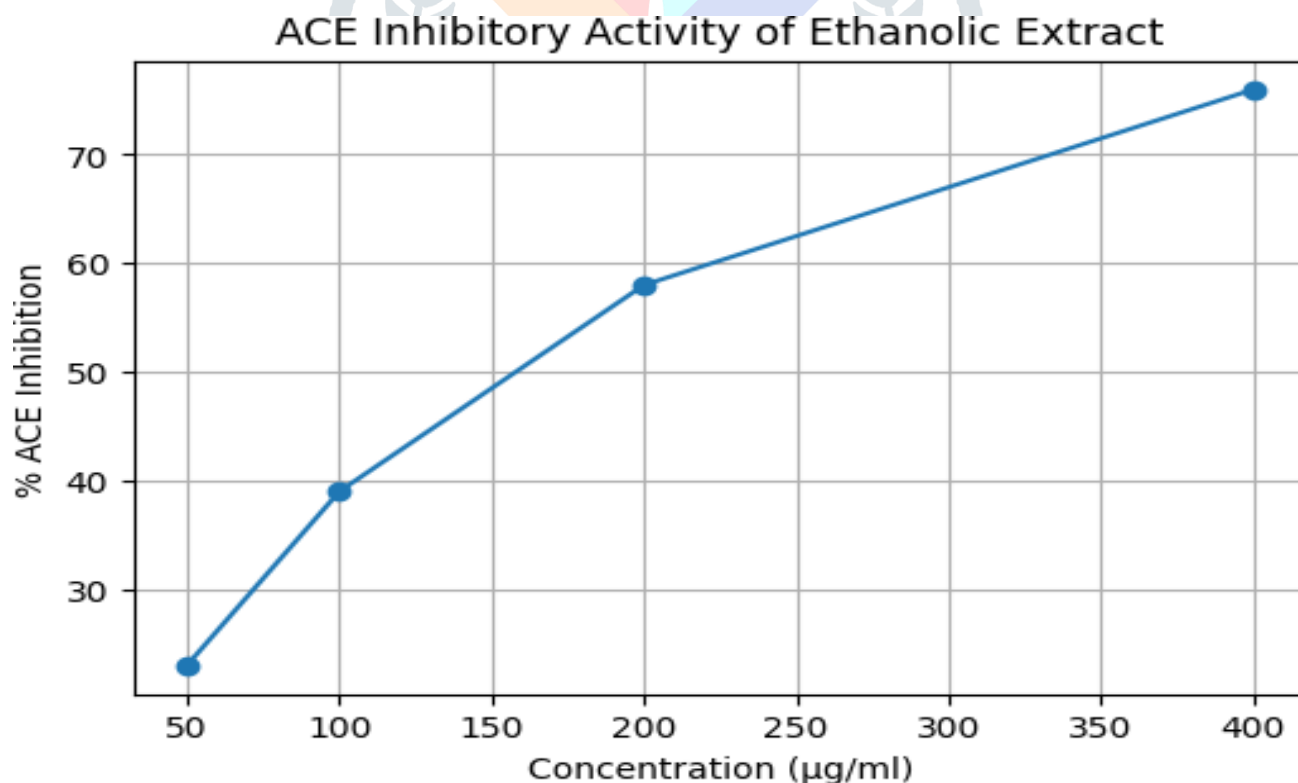
The results show concentration dependent ACE Inhibitory activity for ethanolic and aqueous extracts of averrhoa carambola. The ethanolic extracts shows more ace inhibition as compared to aqueous extracts, hence maximum inhibition shows at 400 $\mu\text{g/ml}$ is 76%.

The standard drug used was captopril and its activity show 88% inhibition at 100 $\mu\text{g/ml}$ concentration.

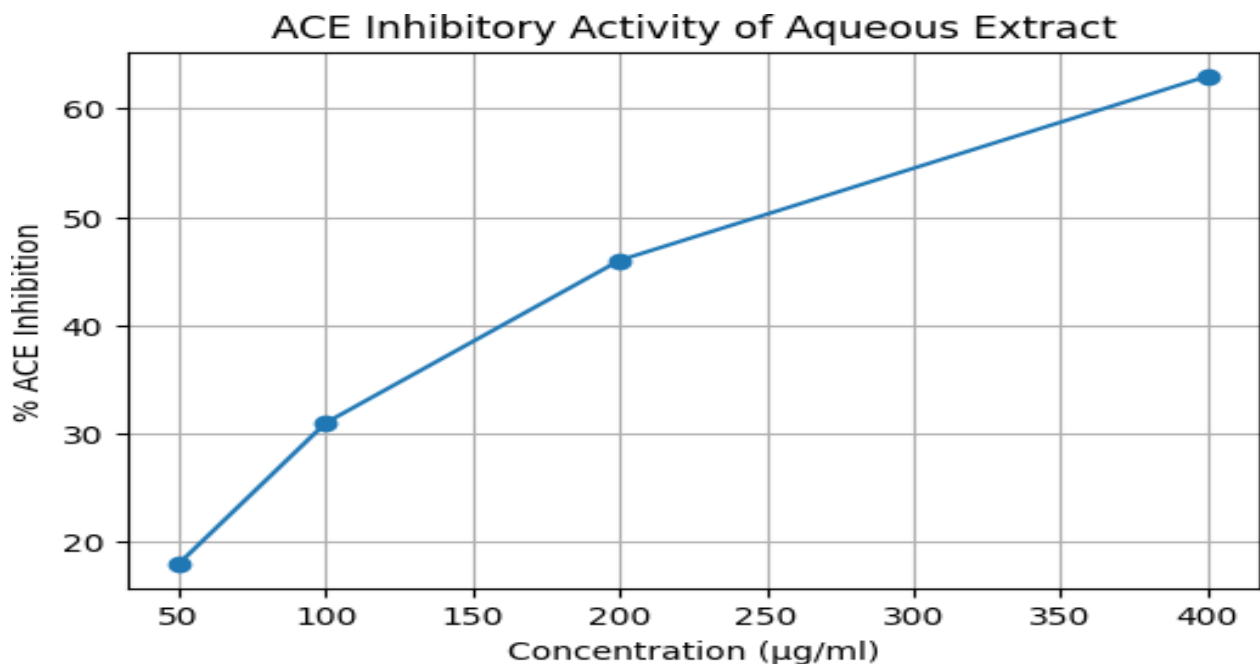
Graphs

A) For Ethanolic Extract

Graph 1: ACE Inhibitory Activity of Ethanolic Extract

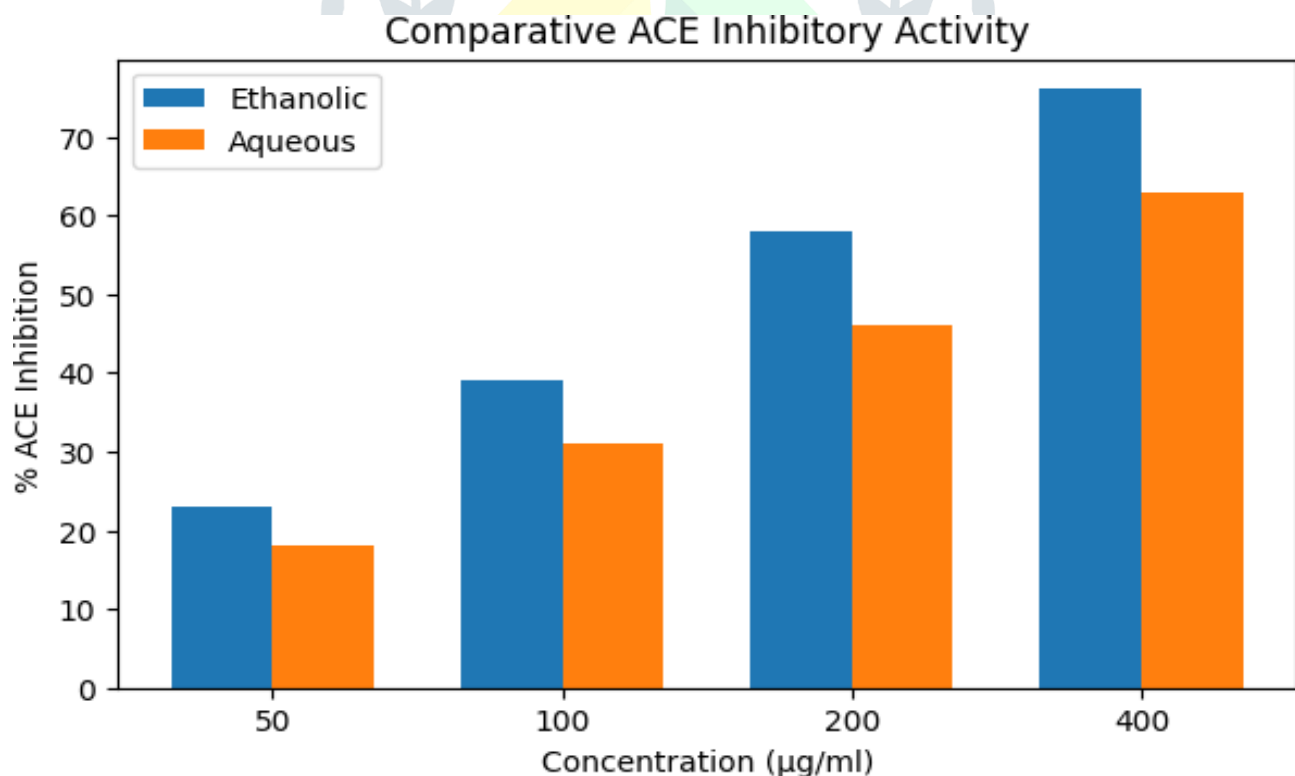


This graph represents concentration dependent elevation in ACE inhibitory activity of ethanolic extract of Averrhoa Carambola. Maximum inhibition was shown at 400 $\mu\text{g/ml}$, which indicates anti-hypertensive potential of extract.

B) For Aqueous Extract**Graph 2: ACE Inhibitory Activity of Aqueous Extract**

The aqueous extract of averrhoa carambola also exhibited ACE inhibitory activity with increase in concentration.

The inhibitory effect of aqueous extract was comparatively less than the ethanolic extract.

C) For Comparative ACE Inhibitory Activity**Graph 3: Comparative ACE Inhibitory Activity of Averrhoa carambola Extract**

This graph shows concentration dependent ACE Inhibitory Activity of both ethanolic and aqueous extracts of averrhoa carambola.

5. CONCLUSION

The present study was carried out to evaluate the in vitro antihypertensive potential of *Averrhoa carambola* fruit extracts. Ethanolic and aqueous extracts were prepared by the Soxhlet extraction method. The percentage yield was 12.4% for the ethanolic extract and 8.6% for the aqueous extract. The results showed that both solvents were able to extract different constituents from the plant material.

Preliminary phytochemical screening showed the presence of several important phytochemical groups. These included alkaloids, flavonoids, tannins, glycosides, saponins, phenolic compounds, carbohydrates, and proteins. The presence of these constituents indicates that *Averrhoa carambola* contains a diverse range of phytochemicals. Some of these compounds are known to possess antioxidant and other biological activities. The ACE inhibitory assay showed activity in both ethanolic and aqueous extracts. The inhibition increased with increasing extract concentration. The ethanolic extract showed greater ACE inhibitory activity than the aqueous extract at the tested concentrations. The highest inhibition was observed at 400 µg/mL. This finding indicates that the ethanolic extract may contain a higher amount of constituents capable of inhibiting ACE under the experimental conditions.

Captopril was used as the reference standard in the assay. The activity of captopril was higher than that of both plant extracts. Therefore, the plant extracts cannot be considered equivalent to the standard drug based on the present results. However, the ACE inhibitory activity observed in the extracts provides a basis for further investigation of *Averrhoa carambola* as a source of bioactive compounds.

The phytochemical results may also help explain the observed biological activity. Flavonoids and phenolic compounds are of particular interest because they are associated with antioxidant activity. Antioxidant constituents may help reduce oxidative stress and may support normal vascular function. However, the present study cannot establish which individual compound was responsible for the observed ACE inhibition.

The findings also suggest that the extraction solvent may influence the biological activity of the plant extract. The higher activity observed with the ethanolic extract may be related to differences in the solubility and extraction of phytochemical constituents. Further chemical analysis is required to confirm this relationship.

The study has some limitations. The investigation was performed using an in vitro model. Therefore, the results cannot be directly applied to clinical treatment of hypertension. The absorption, metabolism, bioavailability, toxicity, and effective dose of the active constituents were not determined. The individual compounds responsible for ACE inhibition were also not isolated.

Further research should focus on the separation and identification of the active phytoconstituents. Advanced analytical techniques can be used for their characterization. Mechanistic studies may help explain their effect on ACE and other pathways involved in blood pressure regulation. In vivo studies should also be performed to evaluate efficacy and safety. Pharmacokinetic and toxicity studies would provide additional information about the therapeutic potential of the extracts.

In conclusion, the present findings demonstrate that *Averrhoa carambola* fruit extracts possess measurable in vitro ACE inhibitory activity. The ethanolic extract showed greater activity than the aqueous extract under the conditions tested. The presence of several phytochemical groups and the observed antioxidant-related potential provide a scientific basis for further investigation. However, additional pharmacological and clinical studies are required before *Averrhoa carambola* can be considered for therapeutic use in hypertension.

Overall, the study provides preliminary evidence that *Averrhoa carambola* may serve as a useful source of naturally occurring compounds with ACE inhibitory activity. The findings can support future research aimed at identifying active constituents and developing standardized plant-based preparations for cardiovascular research.

6. Future Prospects

The following future studies can help establish the antihypertensive potential of *Averrhoa carambola* more clearly:

S.No.	Future Area	Suggested Work	Expected Outcome
1	Isolation of active compounds	Separate and identify the compounds responsible for ACE inhibition.	Identification of potential active constituents.
2	Advanced phytochemical analysis	Use HPLC, LC-MS/MS, GC-MS, or similar techniques for compound profiling.	Detailed chemical profile of the extract.
3	ACE inhibition studies	Study ACE inhibition at different concentrations and determine IC_{50} values.	Better understanding of inhibitory potency.
4	Antioxidant studies	Evaluate the extracts using different antioxidant assays.	Better assessment of antioxidant potential.
5	Mechanistic studies	Investigate the effect of the extract on ACE and other pathways involved in blood pressure regulation.	Understanding of the possible mechanism of action.
6	In vivo studies	Evaluate the extracts in suitable experimental animal models.	Assessment of antihypertensive activity under biological conditions.
7	Toxicity studies	Conduct acute and repeated-dose toxicity studies.	Information on safety and tolerability.
8	Pharmacokinetic studies	Study absorption, distribution, metabolism, and elimination of active compounds.	Information on bioavailability and exposure.
9	Dose optimization	Evaluate different doses of the most active extract or isolated compound.	Identification of an appropriate experimental dose.
10	Standardization	Develop standardized extract preparation and quality-control parameters.	Better reproducibility between batches.
11	Drug-herb interaction studies	Examine possible interactions with commonly used antihypertensive medicines.	Information regarding combined use and safety.
12	Clinical evaluation	Conduct well-designed clinical studies after adequate preclinical evidence.	Confirmation of efficacy and safety in humans.
13	Formulation development	Develop a suitable standardized herbal formulation from the active extract.	Improved stability, dose accuracy, and pharmaceutical application.
14	Long-term safety assessment	Evaluate prolonged use through suitable preclinical and clinical studies.	Better understanding of long-term safety.

References

1. World Health Organization. Global report on hypertension: The race against a silent killer. Geneva: World Health Organization; 2023.
2. Unger T, Borghi C, Charchar F, et al. 2020 International Society of Hypertension Global Hypertension Practice Guidelines. *Hypertension*. 2020;75(6):1334–1357.
3. Whelton PK, Carey RM, Aronow WS, et al. 2017 ACC/AHA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults. *Hypertension*. 2018;71(6):e13–e115.
4. Oparil S, Acelajado MC, Bakris GL, et al. Hypertension. *Nature Reviews Disease Primers*. 2018;4:18014.
5. Rimoldi SF, Scherrer U, Messerli FH. Secondary arterial hypertension: when, who, and how to screen? *European Heart Journal*. 2014;35(19):1245–1254.
6. Carey RM, Calhoun DA, Bakris GL, et al. Resistant hypertension: detection, evaluation, and management. *Hypertension*. 2018;72(5):e53–e90.
7. Montezano AC, Touyz RM. Reactive oxygen species and endothelial function—role of nitric oxide synthase uncoupling. *Circulation Research*. 2012;110(11):1464–1474.
8. Santos RAS, Oudit GY, Verano-Braga T, Canta G, Chappell MC. The renin–angiotensin system: going beyond the classical paradigms. *American Journal of Physiology-Heart and Circulatory Physiology*. 2019;316(5):H958–H970.
9. Turner AJ, Hooper NM. The angiotensin-converting enzyme gene family: genomics and pharmacology. *Trends in Pharmacological Sciences*. 2002;23(4):177–183.
10. Atanasov AG, Zotchev SB, Dirsch VM, Supuran CT. Natural products in drug discovery: advances and opportunities. *Nature Reviews Drug Discovery*. 2021;20:200–216.
11. Wachtel-Galor S, Benzie IFF. Herbal medicine: an introduction to its history, usage, regulation, current trends, and research needs. In: Benzie IFF, Wachtel-Galor S, editors. *Herbal Medicine: Biomolecular and Clinical Aspects*. 2nd ed. Boca Raton: CRC Press; 2011.
12. Muthu N, Lee SY, Phua KK, Bhore SJ. Nutritional, medicinal, and toxicological attributes of *Averrhoa carambola* L. (Oxalidaceae): a review. *BioMed Research International*. 2016;2016:1–9.
13. Shui G, Leong LP. Analysis of polyphenolic antioxidants in star fruit using liquid chromatography and mass spectrometry. *Journal of Chromatography A*. 2004;1022(1–2):67–75.
14. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. *Journal of Nutritional Science*. 2016;5:e47.