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PHYTOCHEMISTRY, ANTI-OXIDANT, ANTI-INFLAMMATORY AND ANTI-MICROBIAL POTENTIAL OF AVERRHOA CARAMBOLA (STAR FRUIT) EXTRACT

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Abstract

This study evaluated the phytochemistry, anti-oxidant, anti-inflammatory and anti-microbial potential of *Averrhoa carambola* (star fruit) extract.The existence of many phytochemistry elements, including carbs, saponins, the compounds flavonoids, the compounds quinones, the terpenoids and steroids, was demonstrated by the Methanol *Averrhoa carambola* fruit extract (ACFE) apart from phenol compounds, glycosides, tannins, and alkaloids. In the DPPH test, the methanolic ACFE extract demonstrated a notable level of antioxidant properties. When comparison to the common medication Diclofenac, the methanolic ACFE displayed good anti-inflammatory properties using the Albumin denatured state method. The test against pathogenic bacterial strains involved various concentrations of methanolic ACFE (50, 100, and 150 mg/mL) and they all showed good activity.

Keywords: *Averrhoa carambola*, anti- oxidant, anti-inflammatory, anti-bacterial.

1. Introduction

In the tropical and subtropical regions, where they are principally distributed, the oxalidaceae family contains seven genera and over two hundred species [1]. Too important of nutritional requirements has been brought to the attention of consumers through extensive publicity and Campaigns aimed at promoting healthy lifestyles were undertaken by the health agencies [2, 3]. In multiple countries [4, 5] it is considered a herb and can either be eaten raw or used in preparation of juices, salads, or pickles. It can be utilized to clean utensils as it assists in removing rust, *Starfruit* consumption has been linked to nephrotoxicity and neurotoxicity in literature through case reports and series [6]. Vitamins and nutrients can be found in abundance in star fruits. Antioxidant compounds like vitamin C, β-carotene, and gallic acid are abundant in star fruits [7, 8, 9].

Moreover, it has reduced-calorie and an extensive fiber content, both of which may help with insulin regulation [10, 11, 12] and therapeutic qualities and phytonutrients of native tropical fruits with economic development potential. Two common inflamed skin diseases in the population are psoriasis and atopic dermatitis; however, the existing treatments for these problems are often ineffective and difficult to tolerate. Conventional folk medicine has employed the Asian tree *Averrhoa carambola* L. (Oxalidaceae) to treat a range of skin ailments. Because of its unusual shape, *Averrhoa carambola* L. (Oxalidaceae), often known as local area name referred as pulichapalam. It is a little, multistemmed, slowly growing evergreen tree that can reach heights of 4-7 m or, in rare cases, 10 m. The leaves are pinnate, spirally organized, 15–25 cm long, In India, fever can be treated by consuming the mature fruit. The leaves as well as the fruit of *Averrhoa carambola* are commonly employed in Ayurvedic to treat a variety of conditions, including pitta, impaired kapha and pruritis.

Antioxidants are substances that can stop and undo harm from too many free radicals. To enhance the quality of sleep and the functioning of the brain, *Averrhoa* are commonly used in alternative medicine. New research reveals that these effects may be caused by their special antioxidants. *Averrhoa* may improve immune function and inhibit the spread of cancerous cells. Reduced levels of free radicals and inflammation can aid in the prevention of chronic conditions like type 2 diabetes. The vitamin C-rich *Averrhoa* fruit is also known to have potent anticancer effects. *Averrhoa* fruit is delicious and tiny. They taste like dates when dried and have a chewy texture.

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Although loquats are frequently grown from seeds, commercial plantings typically use grafted trees of superior types. Shield budding and cleft grafting are the two methods used to reproduce the tree; loquat seedlings or quince rootstocks grown from cuttings can be used, the latter if a miniature tree is required [13,14,15]. The blossoms are relatively vulnerable to fire blight, but the trees are resistant to the majority of illnesses and insect pests. Hence, considering the above facts in view, this study evaluated the phytoconstituents of *Averrhoa carambola* fruit extracts with reference to their anti-oxidant, anti-inflammatory and anti-bacterial potential.

2. Experimental

2.1. Preparation of aqueous and solvent *A. carambola* fruit extracts:

The stored fruit powder of *Averrhoa carambola* (10 g) was extracted with 100 ml of methanol. After the extraction process, the solvents were removed by soxhlet method and evaporated by open air at 40°C to obtain crude extract and stored in beaker.

2.2. Phytochemical screening aqueous and solvent *Averrhoa carambola* fruit extracts:

Extracts Phytochemical screening of *Averrhoa carambola* fruit extracts were assessed by standard method as described by Gayathri and Jayaprakash (16).

Test for Tannins: One ml. of the fruit extract was added to 1 ml. 5% ferric Chloride Formation of dark blue or greenish black indicates the presence of tannins.

Test for Quinones: One ml. of the fruit extract was added to I ml. conc. Sulphuric acid. Formation of red colour indicates the presence of quinones.

Test for Flavonoids: One ml of the fruit extract was added to 1 ml. 2N sodium hydroxide. Formation of yellow colour indicates the presence of Flavonoids.

Test for Alkaloids: One mL of the fruit extract was added to 2 ml conc. HCI. Then, few drops of Mayer's reagent were added. Presence of green colour or white precipitate indicates the presence of alkaloids.

Test for Glycosides: One mL of the fruit extract was added to 3 mL Chloroform and 10% ammonium solution. Formation of pink colour indicates the presence of glycosides.

Test for Terpenoids: One mL of the fruit extract was added to 2 mL Chloroform along with cone, sulphuric acid. Formation of red brown Colour at the interface indicates the presence of terpenoids.

Test for Phenols: One mL of the fruit extract was added to 2 ml. distilled Water followed by few drops of 10% $FeCl_3$. Formation of blue/green colour indicates the presence of phenols.

Test for Steroids: One mL of the fruit extract was added to 2 ml. chloroform and 1 mL sulphuric acid. Formation of reddish-brown ring at interface indicates the presence of steroids.

2.3. Antibacterial activity of methanolic Averrhoa carambola extracts:

Aqueous and solvent extracts of *Averrhoa carambola* fruit were tested against pathogenic bacterial strains namely *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.* The bacterial cultures were grown in Mueller Hinton Agar and Broth (Himedia) (17). Antibacterial activity was measured using diffusion disc plates on agar, About 0.1 mL of each culture of bacteria was spread on agar plate surfaces. For antibacterial assay, all bacterial strains were grown in Mueller Hinton Broth (Hi media) for 24 hours at 37°C and plated on Mueller Hinton Agar (Hi media) for agar diffusion experiments. Paper discs (6 mm in diameter) were placed on the agar medium to load leaf extracts (100 µL) of *Averrhoa carambola*. Inhibition diameters were measured after incubation for 24 to 48 hours at 37°C. Blanks of solvent only (processed in the same way), were also tested for antibacterial activity.

2.4. Antioxidant activity of *Averrhoa carambola* fruit extracts:

The antioxidant activity of methanolic extract of *Averrhoa carambola* was determined by following and Shi *et al.* (18). About 100 µL of fruit extracts of *Averrhoa carambola* were taken in the microtiter plate, 100 µL of 0.1% methanolic DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink was considered as strong and weak positive respectively. The antioxidant positive samples were subjected for further quantitative analysis.

2.4.1. Free radical scavenging activity of Averrhoa carambola fruit extract:

The antioxidant activities were determined using DPPH, (Sigma-Aldrich) as a free radical. Fruit extract of 100 µL were mixed with 2.7 mL methanol and then 200 µL of 0.1% methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control (16). Subsequently, at every 5 minutes interval, the absorption maxima of the solution were measured using a UV double beam spectra scan at 517 nm. Free radical scavenging activity was calculated by the following formula:

% DPPH radical scavenging = [(Abs.of control-Abs.of test Sample) / (Abs. of control)] x 100.

2.5.Anti-inflammatory activity by egg albumin denaturation assay:

Inhibition of egg albumin denaturation was determined using the method prescribed by Chandra *et al.* (17). Phosphate buffer saline (pH 6.4), 8 g of sodium chloride (NaCl), 0.2 g of potassium chloride (KCl), 1.44 g of disodium hydrogen phosphate (Na_2HPO_4), and 0.24 g of potassium dihydrogen phosphate (KH_2PO_4) were dissolved in 800 ml of distilled water was prepared. The pH was adjusted to 6.4 using 1N hydrochloric acid (HCl) and made the volume to 1000 mL with distilled water. About 2.8 mL of phosphate buffer (pH 6.4) and 0.2 mL of egg albumin were incubated with various concentrations (10, 20, 30, 40 and 50 µg/mL) of test samples and standard drug Diclofenac sodium (10, 20, 30, 40 and 50 µg/mL) and the samples were incubated at 37°C for 15 minutes and heated at 70°C for 5 minutes. After cooling, the absorbance of the above solutions was measured using ultraviolet visible spectrophotometer at 660 nm. The percentage inhibition of protein denaturation was calculated using the following formula.

Percentage inhibition = (Abs control − Abs sample)/ Abs control × 100

Table 2. Antibacterial activity of methanolic *Averrhoa carambola* fruit extract.

S.No.	Organism	DMSO	Gentamycin	Zone of inhibition		
				50 µg/mL	100 µg/mL	150 µg/mL
1.	*Klebsiella pneumoniae*	-	27 mm	16 mm	20 mm	23 mm
2.	*Escherichia coli*	-	24 mm	20 mm	22 mm	24 mm
3.	*Staphylococcus aureus*	-	27 mm	18 mm	20 mm	24 mm
4.	*Enterococcus* sp.	-	34 mm	15 mm	21 mm	24 mm

3. Results and Discussion

3.1. Phytochemical screening of *Averrhoa carambola* fruit extract:

For the sake of human health, secondary metabolites provide essential medicinal qualities. In particular, some of these compounds appear to be capable of preventing and suppressing many types of cancer. Compounds belonging to the carbohydrate, alkaloids, quinones, and steroid families are utilized as medications or dietary supplements to treat or prevent various disorders. *Averrhoa carambola* fruit were gathered from Tirupattur for this investigation. The fruits were thoroughly cleaned in distilled water after being washed with running tap water, and they were then allowed to dry in the open air for about a month at room temperature. In order to be used later, the dried fruit material was thoroughly pulverized into powder and stored in a sterile container. *Averrhoa carambola* fruit powder that had been stored was extracted using 100 mL of each of the two solvents, methanol and chloroform. To get crude extract, the solvents were eliminated following the extraction procedure using air drying and an evaporator set at 40°C. *Averrhoa carambola* fruit extract phytochemical screening was evaluated using a conventional procedure as described by Gayathri and Jayaprakash (13,14). The phytochemical components of fruit extracts from *Averrhoa carambola* are listed in Table 1. Except for alkaloids, glycosides, phenols and tannins, all of the phytochemical components tested were found in the methanol extract compared to all other phytochemicals.

S.No.	Phytochemicals	Solvent (Methanol)
1.	Alkaloids	-
2.	Carbohydrates	+
3.	Flavonoids	+
4.	Glycosides	-
5.	Phenols	-
6.	Quinones	+
7.	Saponins	+
8.	Steroids	+
9.	Tannins	-
10.	Terpenoids	+

'+' – Present; '-'- Absent.

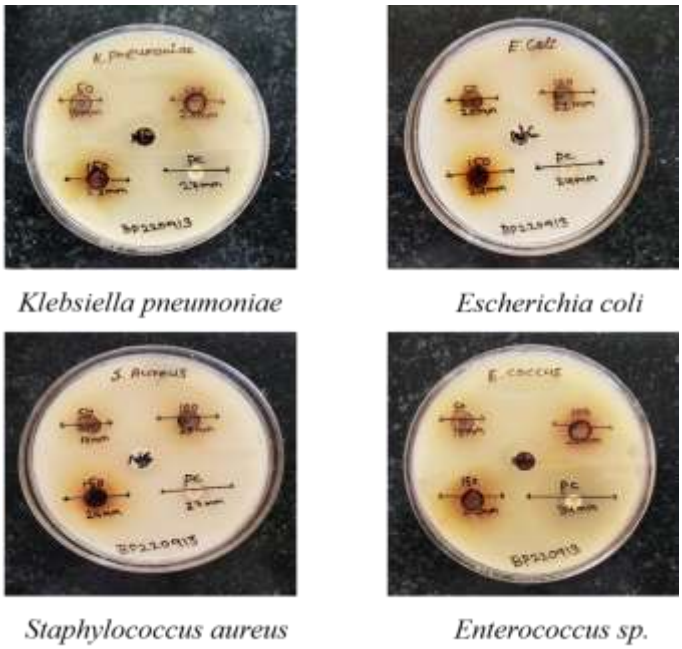
Table 1. Phytochemistry screening of *A. carambola (Star fruit)* methanol extract.

3.2. Antibacterial activity of methanolic Averrhoa carambola fruit extract:

Throughout the beginning of human civilization, people have employed plants as medicine. The use of plants to treat diseases was inevitable, as is clear from the problems with synthetic antibiotics. Many researchers studied *Averrhoa carambola* fruits using a variety of polar chemical solvents, including high (Methanol). Qualitative analysis and antimicrobial activity were examined. The greatest inhibitory zone was against *Enterococcus* sp., *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* were 24 mm, 27 mm, 24 mm, and 27 mm when methanol extracts were compared to the well diffusion

method. *Averrhoa carambola* that had been dissolved in DMSO (300 mg/mL) at doses of 50, 100, and 150 µg/mL had its antibacterial efficacy against the pathogens tested (Figure 1, Table 2).

Figure 2. Antibacterial activity of methanolic *Averrhoa carambola* fruit extract.



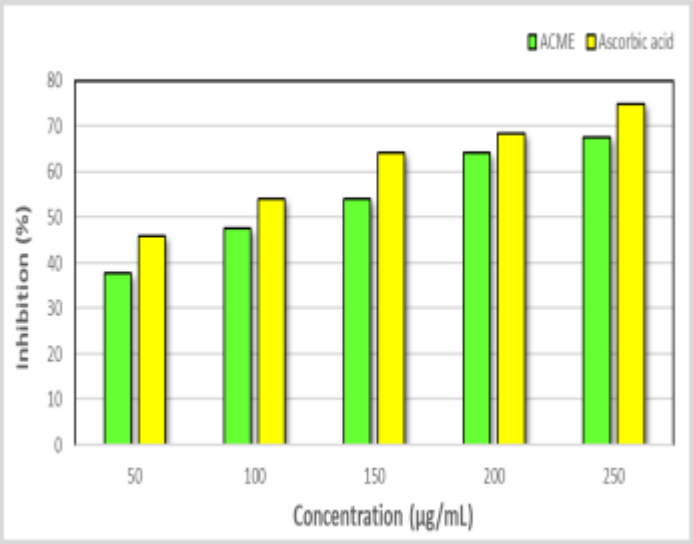
Klebsiella pneumoniae *Escherichia coli*

Staphylococcus aureus *Enterococcus sp.*

3.3. Antioxidant activity of *Averrhoa carambola* fruit extract:

Antioxidants are substances that can stop the chain reactions caused by free radicals. Recently, increased focus has been placed on the therapeutic potential of medicinal plants as antioxidants and re-antioxidants in avoiding tissue damage brought on by oxidative stress. It has been demonstrated that they can bind heavy metal ions, remove free radicals and active oxygen species, and stop lipid peroxidation by inhibiting lipoxygenase. Recently, increased focus has been placed on the therapeutic potential of medicinal plants as antioxidants and re-antioxidants in avoiding tissue damage brought on by oxidative stress. The antioxidant activity was assessed using the DPPH assay. The methanol extract of the *Averrhoa carambola* fruit was shown to have a higher level of free radical scavenging activity suggesting that it has a greater antioxidant potential. According to the DPPH assay, a decreased power potential was shown by an increase in absorbance with concentration. The methanol extract demonstrated strong reducing power used in the investigation; the reported inhibition percentage increased as the concentration increased (Figure 2).

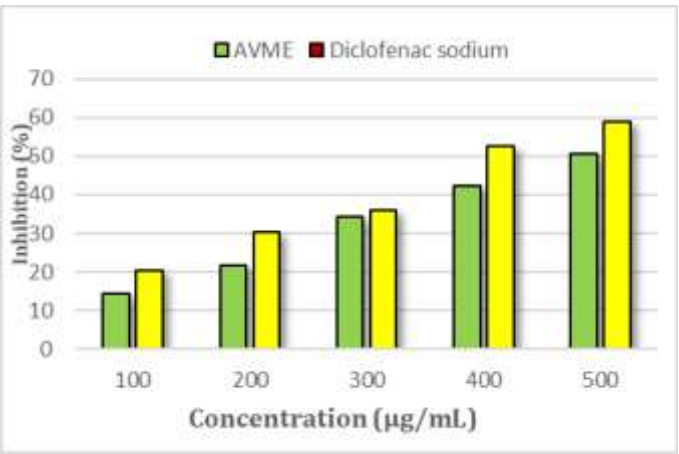
Fig. 2. Antioxidant activity *Averrhoa carambola* fruit extract.



3.4. Anti-inflammatory activity of *Averrhoa carambola* fruit extract:

The inhibition of egg albumin denaturation was assessed spectrophotometrically at 660 nm for *Averrhoa carambola* concentrations between 100-500 µg/mL, Diclofenac, and their interactions. *Averrhoa carambola's* lowest dose of 10 g reduced denaturation of egg albumin protein. There was a progressively rising percentage of denaturation inhibition as *Averrhoa carambola* concentration increased. At various concentrations, diclofenac was found to have a denaturation-inhibiting effect on egg albumin, whereas *Averrhoa carambola* fruit had an identical impact (Figure 3).

Figure 3. Anti-inflammatory activity of *Averrhoa carambola fruit extract*.



4. Conclusions

The existence of many phytochemistry elements, including carbs, saponins, the compounds flavonoids, the

compounds quinones, the terpenoids and steroids, was demonstrated by the Methanol *Averrhoa carambola* fruit extract (ACFE) apart from phenol compounds, glycosides, tannins, and alkaloids. In the DPPH test, the methanolic ACFE extract demonstrated a notable level of antioxidant properties. When comparison to the common medication Diclofenac, the methanolic ACFE displayed good anti-inflammatory properties using the Albumin denatured state method. The test against pathogenic bacterial strains involved various concentrations of methanolic ACFE (50, 100, and 150 mg/mL) and they all showed good activity.

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