Antimicrobial And Phytochemical Analysis Of Averrhoa Carambola And Its Study On Cholesterol-Lowering Effects

K Harinisri, K Madhanasundareswari, R Arthikha

Abstract: Consumption of diets wealthy in fruits and derived food product will bring substantial health edges. Analysis interest focuses on inhibitor and antimicrobial property gifts within the fruit. This study is aimed to grasp the activity of tropical fruit Averrhoa carambola. The binary compound extract of fruit was analyzed for varied phytochemicals and phenolics, flavonoids, alkaloids, and water-soluble vitamin content were gifts in ripe fruit. Antimicrobial activity of the binary compound extract decided by well diffusion technique and inhibition was measured against varied gram-positive and gram-negative organisms. The study conjointly aims to investigate the result of aqueous extract of carambola in reducing the cholesterol level. For this fatty food samples like egg yolk, ghee, chicken fat was treated with extract and calculable by Zak's technique for an amount of our time. Cholesterol lowering results against fatty samples result's ascertained.

Index Terms: Natural therapeutics, Antibiotic resistance, Phytochemical analysis, Antioxidant DPPH test, Dietary fiber, Cholesterol lowering effects, Zak's method.

1 INTRODUCTION

Plants naturally have the potential to stabilize the metabolism of the human body. The medicinal values of plants stand out by not having many side effects compared to other synthetic medicines. Various parts of plants like root, leaves, bark, seeds, and flowers have various medicinal properties [1]. Dietary fiber, minerals, vitamins, Phytochemicals, and antioxidants are richly available in fruits and vegetables according to a recent study [2]. In recent decades antibiotic resistance is a rising problem worldwide [3]. Plant materials act as a source of natural product for maintaining health status and also shows activity against many microorganisms which are survived after chemical or synthetically produced antibiotics. Star fruit also called carambola which is a subtropical and tropical fruit and speculated that Sri Lanka and New Zealand are the origin countries of Star Fruit and these are widely cultivated in parts of South Asia. Bilimbi and Carambola are the two species of genus Averrhoa. The name is derived from the fact of its 5-pointed star-like look when sliced. Star fruit flesh is crunchy, juicy and firm and it is longitudinally 5-6 cm angled, 5-15 cm long and 9 cm wide. Like all fruits, star fruit will look greenish in color in the unripened state, tasting sour and the ripened state looks golden yellow with a mild sweet taste and aromatic odor [4]. The major constitution of Star Fruit is dietary fiber. Fiber is one of the most wanted nutrition to carry out a certain process in the human body. Some of the processes done by dietary fiber are the absorption of dietary cholesterol in the gut, protecting mucous membrane from cancer-causing chemicals in the colon and prevention of constipation Not only in food products, but star fruit is also used for various benefits like removing stains, removal of Iron rust from linen cloth, polishing brass, etc. The potassium oxalate from unripe star fruit can also be used for Dyeing. Star fruit reduces cholesterol absorption in the gut which apparently controls the sugar level in the blood of the human body. Star fruit extract has been evaluated with antimicrobial activity, phytochemical properties and the estimation of flavonoids and antioxidant content in star fruit is done.

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2 MATERIALS AND METHOD

2.1 Collection of sample

The study was carried out for screening the properties present in the Carambola fruit. Fruit sample was collected from local markets in and around Coimbatore region. About 1000g of fruit were collected and stored at 20°C. The handpicked star fruits were washed well-using tap water, twice using distilled water and used further.

2.2 Preparation of extract

About 50g of sample fruit was crushed in mortar-pestle and suspended in 500ml of distilled water. Extraction was done at 70°C for 15 to 30 minutes followed by filtering of the extracts using whatmann No: 1 filter paper. Extracts were then evaporated at 45°C and stored [5].

2.3 Antimicrobial activity

2.3.1 Test organisms

Antimicrobial property of fruit sample was evaluated using different bacterial strains like Bacillus cereus, Escherichia coli, Klebsiella pneumonia, Pseudomonas sp, and Staphylococcus aureus. The isolates were maintained using nutrient agar medium and sub-cultured regularly. The culture was incubated at 35-37°C for 24 hours.

2.3.2 Well diffusion bioassay
Standardized inoculum suspension (0.1ml) of each bacterial strain was spread on nutrient agar plates with sterile swabs. The agar plates were punched with a sterile cork borer size of 6mm and different concentrations of 25µl, 50µl, 75µl, 100µl of the sample was added with a micropipette. Plates were allowed to stand at room temperature for 30min and incubated at 37°C for 24 hours. After the incubation zone of inhibition was measured (mm) [6].

2.4 Phytochemical analysis
Test for alkaloids (Mayer's test): 1ml of the sample treated with few drops of Mayer's reagent. Turbidity or creamy precipitation indicated the presence of alkaloids.Test for Amino acid and Proteins (Ninhydrin test): To a small amount of extract few drops of ninhydrin reagent was added. Then the mixture was heated in a water bath for 2-3 minutes. Yellow color indicated the presence of amino acids. A bluish-blackish color indicated the presence of proteins.Test for carbohydrates (Benedict's test): 1ml of the sample was treated with 2ml of Benedict's reagent. The color change of blue or green or orange or red indicates the presence of carbohydrates. Test for Flavonoids: To 5ml of ammonia solution 1ml of sample and few drops of concentrated sulphuric acid was added. The appearance of yellow color indicated the presence of flavonoids. Test for fixed oils and Lipids: A small amount of extracts was singly ironed between 2 filter papers, and allowed to dry. The look of a grease spot on the paper once discovered below direct daylight indicated the presence of oils and lipids.Test for phenol: An aliquot of the extract was mixed with 5ml of Folins-ciocalteu reagent and 4ml of sodium carbonate. The tubes were vortexed for 15 seconds and allowed to stand for 30 min at 40°C for color development. An appearance of a blue color indicates the presence of phenols. Test for Reducing agents: A little amount of Fehling's reagent was added to the aqueous extract, and the mixture was boiled for 2 min. A brick red color indicated the presence of reducing agents.Test for Saponins (Foam test): The extract was diluted with distilled water and shook well in a graduated cylinder for 15 min. The persistent foam to a length of 1cm indicates the presence of saponins. Test for Tannins (Lead acetate test): To 2-3ml of extract, 0.5ml of 1% of lead acetate was added and the formation of a white precipitate indicates the presence of tannins and phenolic compounds. Test for Trepenoids: To 5ml of extract about 2ml of chloroform and 3ml concentrated sulphuric acid was added. The appearance of reddish-brown color at interface indicated the presence of trepenoids.

2.5 Nutrient content

2.5.1 Ascorbic acid content: To 20ml of the sample 200ml of distilled water and 1ml of 0.5% of starch indicator solution was added. The sample was titrated with iodine solution which includes potassium iodide and iodine and endpoint were identified. The permanent trace of dark blue-black indicated the presence of ascorbic acid and total content in the sample was estimated.

2.5.2 Carbohydrate content: Estimation of carbohydrate was done using glucose as standard had taken in the range between 100 to 1000mg/ml. Distilled water was used as a diluent. To 1ml of a sample in a tube 1ml diluents, 1ml DNSA (Dinitrosalicyclic acid) were added. The tubes were kept in boiling water bath for 10min, and made up to 10ml using diluents. Then absorbance measured at 530nm using a spectrophotometer. Plotting the graph optical density vs. concentration of glucose and sample value was interpolated using a standard curve.

2.5.3 Protein content: Bovine serum albumin used as standard ranged between 100 to 1000 mg/ml. As distilled water used as diluents, 1ml of sample and 5ml of alkaline copper sulfate was added and incubated at room temperature for 10 min. After incubation added 0.5ml of folin ciocalteau reagent (1:1 diluted) and mixed at once. Samples were incubated at room temperature for 30 min and absorbance measured at 660nm using a spectrophotometer. Plot the graph optical density vs. concentration and protein content was calculated.

2.6 Antioxidant assay

2.6.1 DPPH free radical scavenging activity: The antioxidant or free radical scavenging activity of the extracts was measured on the basis of a decrease in the absorbance of the ethanolic solution of stable DPPH. DPPH means 1,1- diphenyl-2-picrylhydrazyl free radical exhibit a dark purple color at absorbance 530nm [7]. After scavenging light yellow color was observed. Serially about 1ml to 5ml of the sample was taken and made up to 9ml with pure ethanol. From the diluted aliquots, 1ml was taken and 3ml of DPPH (25mg/L) solution was added and made up to 9ml with ethanol. The mixture was jolted smartly and unbroken at room temperature for thirty minutes in dark and absorbance was measured. Scavenging activity was calculated as the percentage inhibition (%) using the following formula:

$$\% \text{ DPPH anti-radical activity} = \frac{(\text{control absorbance} - \text{Sample Absorbance}) \times 100}{\text{Control absorbance}}$$

2.7 Total flavonoid content
1ml of the sample was mixed with 4ml of distilled water and subsequently with 0.3ml of 10% of sodium nitrite solution. After 5 min, 0.3ml of 10% aluminium chloride solution and 2ml of 1% sodium hydroxide was added. Immediately, the absorbance of the solution was measured at 510nm using Gallic acid as standard. A standard curve was prepared and results were expressed in microgram Gallic acid equivalents (mg GAE/g).

2.8 Total Phenolic content
1ml of the sample was mixed with 5ml of distilled water and 0.5 ml of Folins-ciocalteu reagent was added. The mixture incubated for 5 min at room temperature. After incubation 1.5ml of 20% of sodium carbonate was added and volume was made up to 10ml using distilled water, mixed and allowed to stand for 120 min at room temperature. The blue color change was observed and the absorbance read at 760nm. Gallic acid (100-1000 mg/L) was used as standard and TPC expressed in terms of milligram Gallic acid equivalent per 100-gram dry matter (mg GAE/100g dm) [8].

2.9 Estimation of reducing cholesterol activity

To a standard solution (100-1000 mg/ml) with different fatty food samples like ghee, egg yolk and artificial cholesterol were made up to 5ml with a ferric chloride-acetic acid solution (0.05% of Fecl3 in 100ml of acetic acid) was added. 3ml of concentrated sulphuric acid is added and mixed well. An equal amount of 1ml fruit extract was added and incubated at room temperature for 20-30 minutes. Blank was prepared using 5ml of ferric chloride-acetic acid and 3ml concentrated sulphuric acid along with 1 ml of fruit extract and intensity was measured at 560nm. After incubation for 24 hours, the reducing cholesterol content was estimated and determined graphically [9]. Statistical analysis: For all tests, three replicates were done and the mean values and standard deviations were determined.

3 RESULT

3.1 Preparation of extract

The fruit was initially washed in water and was cut into small cubes. The cut cubes were crushed with mortar and pestle and further subjected for extraction at 70°C for 30 min. Hence they are non-polar in nature the contents present in the fruit were dissolved in aqueous diluents and extract were filtered and stored at 20°C.

3.2 Antimicrobial activity

The well diffusion assay showed that the fruit extracts have different degrees of bacterial growth inhibition, depending on the strains. Fruits of Averrhoa carambola showed better antimicrobial activities.

TABLE 1
RESULTS FOR ANTIMICROBIAL ACTIVITY

S.No	Organisms	Dilutions (mm)			
		25µl	50µl	75µl	100µl
1	Bacillus cereus	13	15	17	20
2	Escherichia coli	-	-	-	20
3	Klebsiella pneumoniae	-	10	11	13
4	Pseudomonas sp	11	15	16	18
5	Staphylococcus aureus	11	12	14	17

Averrhoa carambola found to have potent antibacterial activity against medically important bacterial strain. The fruit extract showed good antimicrobial activity against Bacillus cereus and was less effective against E.coli, were both showed a zone of inhibition about 20mm and 9mm respectively.

3.3 Phytochemical assay

The edible parts of the fruits were analyzed for different phytochemicals.

TABLE 2
RESULTS FOR PHYTOCHEMICAL TEST

PHYTOCHEMICAL TEST	RESULT
Alkaloid (Mayer's test)	+
Amino acids and Proteins (Ninhydrin test)	+
Carbohydrate (Benedict's test)	+
Flavonoids	+
Fixed oils and Lipids	+
Phenol	+
Reducing agents	+
Saponins	+
Steroids	+
Tannins	+
Trepenoids	-

(+) indicates Positive; (-) indicates Negative

The fruit sample had been observed to have certain bioactive phytoelements such as flavonoids, phenols, etc., that were highly nutritive and supports the health status of the human body.

3.4 Nutrient content

3.4.1 Ascorbic acid content: The ascorbic acid was estimated with iodine titration method and titration was done in triplicates, an average value of titrant was obtained. Ascorbic acid estimated in the fruit sample was observed to be 3. 538mg/ml.

3.4.2 Carbohydrate content: DNSA (Dinitrosalicyclic acid, 1959) method was used for the determination of reducing sugars present in the fruit sample. The carbohydrate content present in the fruit sample was calculated using glucose as standard which was taken at different concentrations (100-900mg/ml) and the graph was plotted against optical density at 540nm.

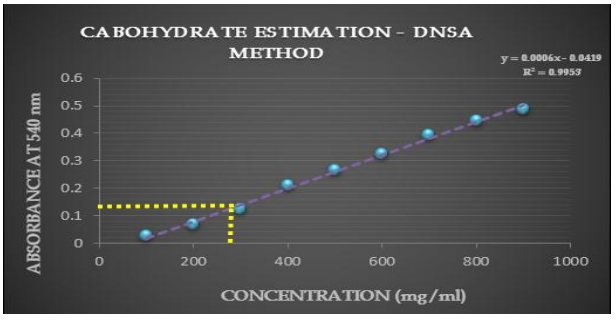

Fig. 1. *The standard graph was plotted by DNSA method with concentration in x-axis and absorbance in y-axis. The carbohydrate content in fruit sample value was interpolated and concentration was determined.*

The sample value was interpolated against the standard graph and the concentration was determined in fig. 1 using absorbance. The carbohydrate content in the fruit sample was about 300mg/ml.

3.4.3 Protein content: Lowry et al method (1951) was used for the determination of protein present in the sample. By using Bovine serum albumin as standard has taken in different concentrations (100-900 mg/ml) graph was plotted against optical density at 660nm. The sample was interpolated against the standard graph and concentration was determined in fig. 2. The protein content in the fruit sample was found to be 110mg/ml.

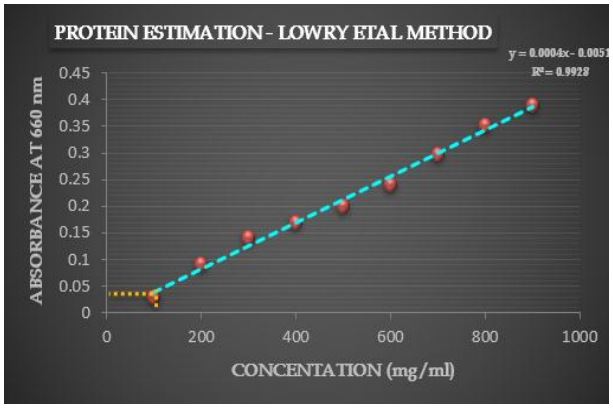

Fig. 2. *The standard graph was plotted using Lowry etal method with concentration and absorbance in x and y-axis. The fruit sample value had been interpolated in standard graph and concentration was determined.*

3.5 Antioxidant assay

DPPH is one of the few stable and commercially available organic nitrogen radicals exhibiting a dark purple color at absorbance 530 nm. When free radicals are scavenged, DPPH will be reduced, producing a light yellow coloration reducing the absorbance. The different concentration of the sample was plotted against the absorbance and inhibition concentration was calculated. When a solution of DPPH is mixed with the sample that can donate a hydrogen atom, which gives rise to the reduced form (Diphenylpicrylhydrazine; non-radical) with the loss of violet color and final color expected to be a residual pale yellow from the picryl group until it present. IC 50 value found to be 24.8 µg/ml for alcoholic extract.

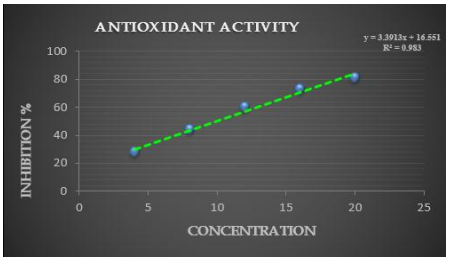

Fig. 3*. The graph was against different concentration of sample with concentration and inhibition percentage in x and y-axis. The antioxidant activity was linearly determined.*

3.6 Total flavonoid content

Flavonoids were estimated using Gallic acid as standard has taken in different concentrations (100-900mg/ml). The absorbance value of the fruit sample was interpolated against the standard and concentration was determined in fig. 4. The flavonoid content in fruit was about 80m g/ml.

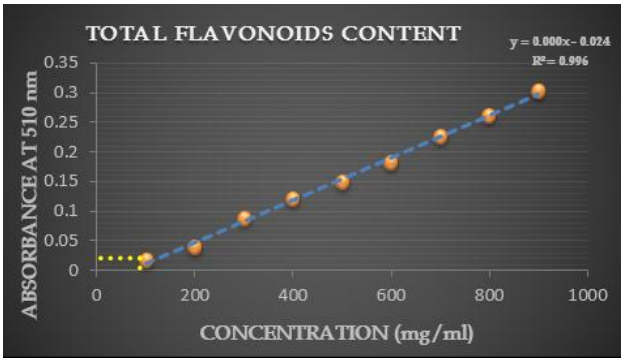

Fig. 4. *The standard graph was plotted with concentration in x-axis and absorbance in y-axis. The total flavonoids present in the fruit sample value was interpolated and flavonoid concentration was determined.*

3.7 Total phenolic content

Gallic acid is used as a standard and taken in different concentrations (100-900m g/ml). Folin's ciocalteau method was used to calculate the total content in the sample. The phenolic content of the fruit sample was interpolated with the standard graph and concentration was determined in fig. 5. The phenolic content in fruit was about 115mg/ml.

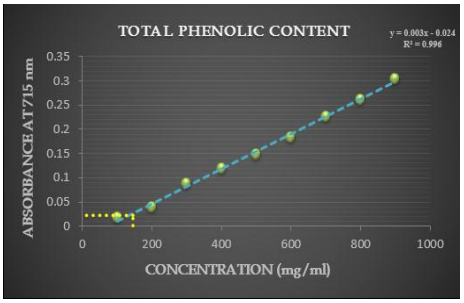

Fig. 5*. The standard graph was plotted with concentration in x-axis and absorbance in y-axis. The total phenolic value in the fruit sample was interpolated and flavonoid concentration was determined.*

3.8 Estimation of reducing cholesterol activity

Zak's method is used for the estimation of cholesterol content. This methodology was modified with fatty food samples like artificial cholesterol, egg yolk and ghee in different concentrations (100-900mg/ml). To that concentration, an equal volume of fruit sample was added and initial absorbance was taken. After 24 hours of incubation, the final absorbance was taken and the obtained values were compared graphically. The cholesterol-reducing nature was estimated by the addition

of fruit samples with fatty food standards and exposed for a certain period (or hours) and the reduction level was determined. The reduction level of cholesterol as compared with the initial and final absorbance that was determined graphically in fig. 6, 7, and 8.

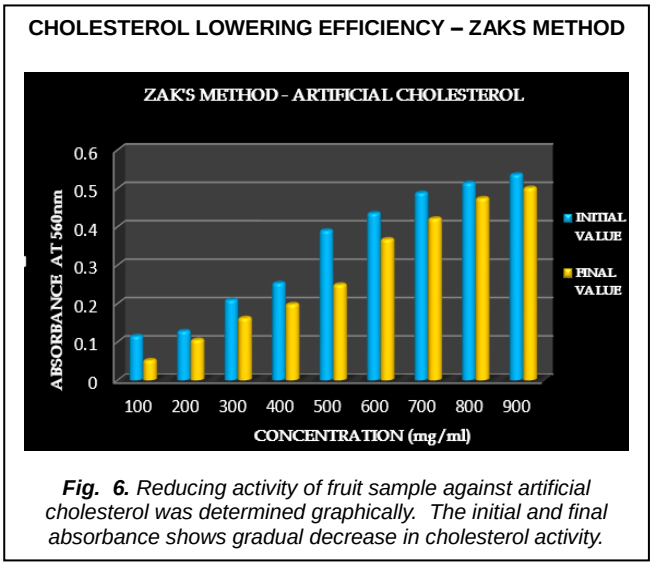

Fig. 6. *Reducing activity of fruit sample against artificial cholesterol was determined graphically. The initial and final absorbance shows gradual decrease in cholesterol activity.*

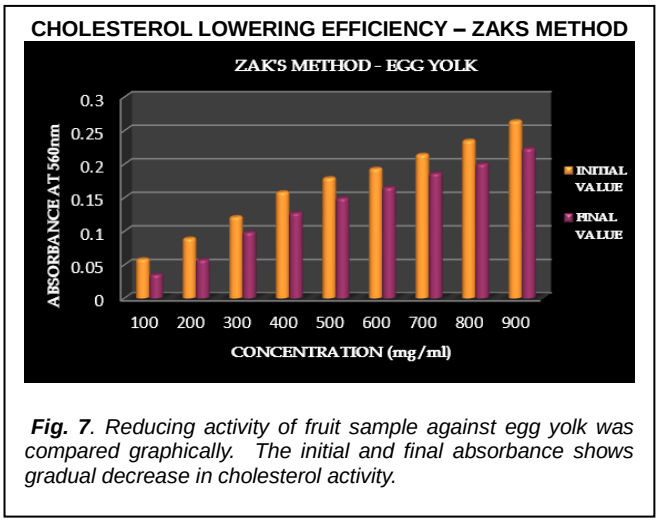

Fig. 7*. Reducing activity of fruit sample against egg yolk was compared graphically. The initial and final absorbance shows gradual decrease in cholesterol activity.*

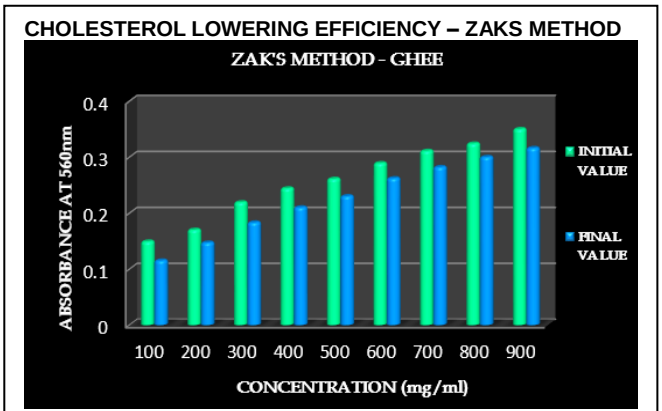

Fig. 8. *Reducing activity of fruit sample against ghee was compared graphically. The initial and final absorbance shows gradual decrease in cholesterol activity.*

4 CONCLUSION

Carambola, a tropical and subtropical fruit along with its peel provides a good amount of dietary fiber which helps in the prevention of absorption of dietary LDL-cholesterol in the gut. This study shows that fruit sample contains active components which inhibits medically important bacterial species. The phytonutrients and nutrition content were abundantly present in the sample. The antioxidant activity was determined by DPPH reducing activity. The dietary fiber within the fruit reduces the sterol sourced sample and remedial importance of fruit was exposition. The fruit acts as another natural therapeutic supply for sterol reduction that may be a major cause for numerous physiological sufferings. This study hastens the natural therapeutic mode that alternates from usual therapeutic ways.

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