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A COMPREHENSIVE STUDY ON ANTIOXIDANT, ANTIBACTERIAL, CYTOTOXIC AND PHYTOCHEMICAL PROPERTIES OF *AVERRHOA CARAMBOLA*

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Abstract: Experiment was conducted on petroleum ether extract of a bark part of *Averrhoa carambola* in order to find out various phytochemicals in this plant and to evaluate this plant's cytotoxicity, ability to impede bacterial growth and ability to work against harmful molecules like free radicals with the help of antioxidants that are present in it. To make sure that the findings are accurate, a number of samples of this plant were collected from different places of Bangladesh. Existence of flavonoid, carbohydrate, glycoside and steroid in the petroleum extract of *Averrhoa carambola* was confirmed by conducting a phytochemical screening. The disc diffusion method, where ten pathogenic bacteria were used, confirmed that the extract is capable of showing good action against pathogenic organisms. But among these ten bacteria, the extract's zone of inhibitions were comparatively larger in cases of *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus megaterium*, which clearly indicates that the extract is able to exhibit remarkable antibacterial action against these bacteria. The cytotoxic action of the extract was measured by using the Brine Shrimp Lethality Bio-assay method. Using this method, the value of LC50 was calculated as 19.95. The extract's phenolic content was estimated as 62.504 mg/g GAE, and the amount of flavonoid in it was 24.107 mg/g of quercetin equivalent. A slight increase in the DPPH radical scavenging activity of *Averrhoa carambola* was observed when the concentration of the extract was enhanced. In comparison to the IC50 value of 11.20µg/mL of ascorbic acid, which was considered as a reference in the test, the IC50 value of the plant extract was estimated as 125.429µg/mL.

Keywords: *Averrhoa carambola*, Phytochemical Screening, Disc Diffusion Method, Antioxidant Activity, Brine Shrimp Lethality Bio-Assay.

INTRODUCTION

Averrhoa carambola, a tropical, drought-resistant plant known commonly as Carambola or Starfruit because of having a shape like a star, is considered as a valuable species in the family Oxalidaceae for its multifaceted medicinal properties. According to historians, Sri Lanka and Maluku islands were the places where cultivation of star fruit was started for the first time. Since then, this evergreen plant has been grown in China, Taiwan, and Malaysia and in countries of Southern Asia[12,8]. In fact, this plant can grow in abundance in any country having a warm climate. The fruit of this plant - which can taste sour in early stage of ripening and very sweet, when fully ripe - has also been used in cooking for a long time, especially to add extra flavor in food items like puddings, stews, tarts and curries. On the other hand, this species has drawn the attention of researchers for its ability to cure diseases and strengthen human immune system. Therefore, this study has been carried out in order to identify and know in more detail about the medicinal qualities that are existent in this particular plant. We found out that *Averrhoa carambola* contains chemical compounds like saponins, steroids, alkaloids, glycosides, flavonoids and carbohydrates, which bear clear testimony to this plant's high medicinal values. According to Materia Medica, it is able to quench thirst, and has been used for long to increase secretion of salivary glands and in the treatment of fever[11]. Moreover, according to Ayurvedic medicine, ripe starfruit helps in digestion and works effectively as a tonic, but biliousness can be caused in case of excessive intake. In India, it is considered as one of the best cooling medicines[6,14]. This fruit also contains antioxidants, the juice can be used as an astringent, especially in cases of hemorrhage, and bleeding hemorrhoids, it works effectively to stop further loss of blood[9]. In Brazil, it has been used as a diuretic to help patients with bladder and urinary problems[11]. The main objectives of this study were to determine the antioxidant,

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antibacterial and phytochemical properties of *Averrhoa carambola* and get a clear view of how toxicity develops in a cell in case of *in vivo* administration of extract or medicinal agent of this plant. In this regard, we used Brine Shrimp Lethality Bioassay, which was proposed by[11] and modified by[17], in order to assess the cytotoxic action of the plant materials.

MATERIALS AND METHODS

Collection of sample and preparation of plant extract:

After properly cutting and slicing, the collected sample of this species was dried in two stages – firstly, in the sun and then by an electric dryer at 60-70◦C. After that, the dry sample was turned into powder by grinding in a mechanical grinder. The powdered sample was preserved in a clean and closed glass container. Later, in order to let the extraction process occur completely, chloroform and ethanol having a total volume of 800 ml was entered into the container and the dry sample was left in this condition for the next 3 days. Then the plant extract was filtered in a sterilized cotton filter and the obtained filtrate was collected in a beaker. The solvent was completely evaporated from the solution by placing the plant extract in a water bath at 60°C. For the next 72 hours, the container was kept airtight and the filtrate that accumulated during this period was concentrated by using a rotary evaporator.

Procurement of chemicals and drugs:

We purchased DPPH (1, 1-diphenyl, 2-picrylhydrazyl), TCA (trichloroacetic acid) and ferric chloride from Sigma Chemical Company of USA, Ascorbic acid from SD Fine Chem. Ltd. of India and Ammonium Molybdate from Merck, Germany.

Presence of phytochemicals:

In order to know clearly about the various chemical constituents of the plant extract, standard techniques were applied at the time of conducting the phytochemical screening. Required chemicals and reagents were also used to testify freshly prepared crude extracts of *Averrhoa carambola.* For example, Mg and HCL were used to confirm the presence of flavonoids. To find out whether tannin was present, ferric chloride and potassium dichromate solutions were used. The plant extract's ability to produce stable foam indicated that saponin was present. Besides, Benedict's reagent was used for detecting reducing sugars and presence of steroids was confirmed by Libermann Burchard reagent. Throughout the experimentation, we carefully observed the change of color at different stages[4].

Antibacterial assay:

To assess the antibacterial activity of the dry sample extract, ten pathogenic bacteria were used as test organisms and disc diffusion method was followed while conducting this antimicrobial assay. BCSIR of Chittagong, Bangladesh gave us the required bacterial strains. 0.5mg/disc of the sample was used to observe the antimicrobial activity of the plant extract and to compare with kanamycin (0.1mg/disc), which was considered as standard. A 10 ml previously sterilized nutrient agar media was used to inoculate the organisms that were used in this test and after fully mixing them with the media, they were quickly moved to a sterile Petri dish with the help of sterile loop. A completely aseptic condition was maintained while performing this task. As the Petri dish was ready, we put the prepared sample along with standard solutions in it and then incubated the plates at 37 °C for about 12 hours. Finally, after letting them go through proper incubation, we observed and measured the area of inhibition surrounding the place where sample solution was applied.

Determination of total phenolic content:

With the help of Folin-Ciocalteu method, the total phenolic content was measured. Folin-Ciocalteu oxidized the extract while sodium carbonate neutralized it[16]. At this stage, blue color was noticed. After 60 minutes, we measured the absorbance at 760 nm by using Gallic acid (GA) as standard. Total phenolic content was expressed as mg GA equivalent/gm of extract.

Determination of total flavonoid content:

To determine the flavonoid content**,** we applied Kumaran and Karunakaran method[7] and used quercetin as standard. 1 mg of plant extract in methanol was mixed with 1 ml of aluminium trichloride in Ethanol (20 mg/ml) and then a drop of acetic acid was added. The mixture was diluted up to 25 ml with ethanol and 40 minutes later, the absorbance was measured at 415 nm. Measurement of blank samples and standard quercetin solution (0.5 mg/ml) in methanol was done under the same conditions.

DPPH radical scavenging activity:

With the help of DPPH, the free radical scavenging capacity of the plant extract was determined [5,1]. A methanol DPPH solution (0.004% w/v) was mixed with serial dilutions (0 to 500µg) of *Averrhoa carambola* extract and after 10 minutes the absorbance was taken at 517nm using spectrophotometer. Ascorbic acid was used as a standard. Finally, after plotting the inhibition curve, IC50 values were found out from it.

Reducing power:

The reducing power of *Averrhoa carambola* extract was found out by using the method of Oyaizu [13]. Different concentration of *Averrhoa carambola* extract in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). For the next 20 min, the mixture was incubated at 50°C. After adding

slight amount of (2.5 ml) of Trichloroacetic acid (10%), the mixture was centrifuged at 3000 rpm for 10 minutes. Next the upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1%) and then the absorbance reading was taken at 700 nm. The Ascorbic acid was reference standard and the blank solution had Phosphate buffer in it.

Brine shrimp lethality bioassay:

At this stage of our study, we used Brine Shrimp Lethality Bioassay to evaluate cytotoxic potential of the extract[10,15]. A tank having a temperature of 37°C and continuous supply of oxygen as well was used to hatch the Brine Shrimp eggs. After 48 hours, the matured nauplii were collected. As per requirement, extract was dissolved in specific volume of pure dimethyl sulfoxide (DMSO) and stock solutions were prepared. After that, 4 ml of seawater was added to each of the vials, and later specific volume of sample was transferred from the stock solution to the vials to obtain final sample concentrations of 010, 20, 40, 60, 80, 100, 200, 300, 400, 500, 600, 800 and 1000µg/ml. Then same volumes of DMSO (as in the sample vials) were taken in the control vials. Using Pasteur pipette 10 living nauplii were put in each of the vials and they were kept in this condition for a day. 24 hours later, we observed their condition, counted the number of nauplii that were alive and calculated the percentage of lethality of Brine Shrimp nauplii for each concentration of the extract.

RESULTS AND DISCUSSION

Phytochemical screening:

After carrying out a thorough phytochemical analysis of the plant extract, we were able to verify whether flavonoids, alkaloids, tannins, saponin, steroids, carbohydrate, antranquinone, protein, glycoside and resin were present or not. For instance, tests confirmed that flavonoid, glycoside, alkaloid, carbohydrates, saponin and steroid were present while tannin, protein and resins were not. The findings have been shown in table 1.

Table.1: Identified chemical constituents of the *Averrhoa carambola*.

Compound name	Plant Parts
	Bark
Alkaloid	Present
Flavonoid	Present
Tannin	Absent
Glycoside	Present
Anthraquinone	Absent
Carbohydrate	*Molisch's Test* Present *Benedict's test* Present
Resin	Absent
Protein	Absent
Saponins	Present
Steroids	Present

Antibacterial assay:

By conducting the antibacterial assay[3,2], we were able to gain valuable knowledge about antibacterial activities of *Averrhoa carambola*. Ten pathogenic bacteria were used to examine the extract's strength of hindering bacteria from colonizing. In this case, the area of inhibition for every bacterium was measured and the results were expressed in millimeter (mm). Kanamycin used as the standard antibiotic to compare the results. The results have been shown in figure 1.

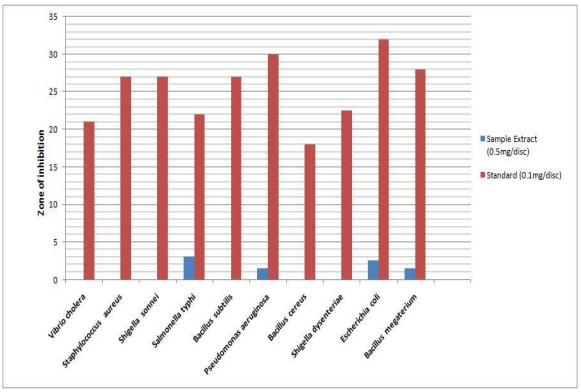

Figure.1: Antibacterial activity ethanol extract of *Averrhoa carambola* stem bark.

The figure indicates that the ethanol extract of *Averrhoa carambola* bark was able to kill some of the bacteria. It is also obvious that the plant extract was able to show antibacterial activity against *S. typhi, P. aeruginosa, E. coli* and *B. megateriu.* On the other hand, the extract could not show good performance in inhibiting the growth of the rest six bacteria, which are *Vibrio cholera, Staphylococcus aureus, Shigella sonnei, Bacillus subtilis, Bacillus cereus* and *Shigella dysenteriae.* In antibacterial screening the plant extract showed average inhibitory zone 1.5-3.00 (mm) and against *S. typhi.* Showed narrow inhibition property.

DPPH radical scavenging activity:

The DPPH radical scavenging activity of *Averrhoa carambola was* shown in figure 2. Where increasing the concentration of the extract, activity was found to increase slightly and the inhibitory capacity of the plant extract was comparatively lower than the ascorbic acid.

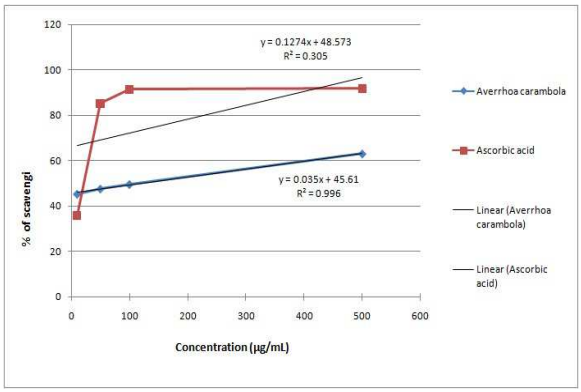

Figure.2: DPPH radical scavenging activity of Chloroform extract of *Averrhoa carambola*.

In DPPH test, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action. Petroleum ether extract of *Averrhoa carambola* showed good DPPH scavenging activity. IC50 value for the plant extracts was 125.429µg/mL. Ascorbic acid was chosen as the reference antioxidant for this test and IC50 value for ascorbic acid was 11.20µg/mL.

Reducing power:

By using the potassium ferricyanide reduction method the reductive capabilities of the plant extract was identified in comparison with ascorbic acid which demonstrated at figure 3. The reducing power of the extracts was moderately strong while increasing dose it shows little increment.

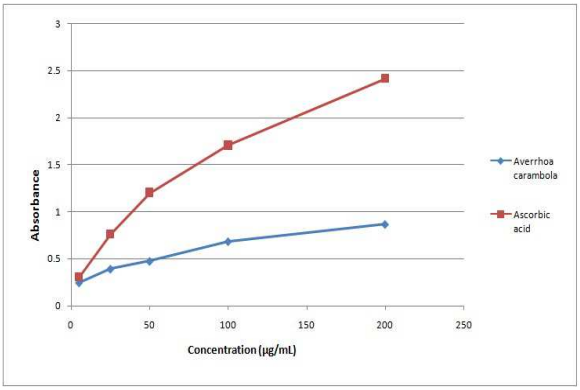

Figure.3: Reducing Power of the chloroform extract of *Averrhoa carambola* vs Standard.

Total phenol and flavonoid content:

The total phenol content and total flavonoid content have been expressed in gallic acid and quercetin equivalents respectively. We got a moderate result (159.6014 mg/g GAE) for the phenolic contents and amount of flavonoid was 28.3377 mg/g quercetin equivalent. Flavonoids and phenolic acids types of poly phenolic compounds in plants contain multiple biological effects, including antioxidant activity which is proven by different studies. Present studies indicate the presence of polyphenolic compound in bark part of *Averrhoa carambola*. It has been found that the antioxidant effect of plant product is generally due to radical scavenging activity of phenolic compounds such as flavonoids, polyphenols, tannins, and phenolic terpenes[9].

Bioassay of brine shrimp lethality:

By applying Brine Shrimp Lethality bioassay, we assessed the plant extract's cytotoxicity. Its cytotoxic action on Brine shrimp nauplii was closely monitored. Here DMSO was used as a solvent. Whether DMSO had any effect on brine shrimp lethality or not, control was used in this study. The control group of brine shrimp nauplii with and without DMSO exhibited no mortality. The number of nauplii died and percent mortality was counted for the subjected extract. The result has been shown in table 2.

Table.2: Brine shrimp Lethality Bioassay for the ethanol extracts of *Averrhoa carambola* bark.

Sample Concentration C (µg/ml)	Log C	No. of viable Shrimps (out of 10)	% Mortality	LC50 (ug/ml)
1000	3.00	0	100	19.95
800	2.9	0	100	
600	2.77	0	100	
500	2.69	0	100	
400	2.6	0	100	
300	2.47	0	100	
200	2.3	0	100	
100	2.0	2	80	
80	1.90	1	90	
60	1.69	1	90	
40	1.30	6	40	
20	1.3	8	20	
10	1	10	0	

LC50 (Lethal Concentration) of ethanol extract was 19.95. Therefore, there is no doubt that ethanol extracts of *Averrhoa carambola* is much toxic to cell. It has been found that here the value of Log C is 1.3.

CONCLUSION

After accomplishing this thorough study on a sample of *Averrhoa carambola*, it became evident to us that though this species does not show very strong antibacterial activity, it possesses good antioxidant property. In our opinion, difference was noticed in antibacterial or antioxidant activity of the plant extracts against different bacteria due to presence of various active phytochemicals like flavonoid, glycoside, alkaloid, carbohydrates, saponnines and steroid. Another important characteristic that we also found out about the plant extract is that it is capable of fighting with free radicals as well as damage that is caused by such radicals. Our study also clearly indicated that, in terms of cytotoxicity, this species is able to show good cytotoxic activity.

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