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EVALUATION OF *IN-VITRO* ANTIOXIDANT AND BRINE SHRIMP LETHALITY ACTIVITIES OF FRUIT EXTRACT OF *AVERRHOA CARAMBOLA* L.

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Keywords:

Averrhoa carambola L., Antioxidant activity, Cytotoxicity, Free radical scavenging, Polyphenols, Flavonoids

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ABSTRACT: Research with plants and plant derivatives are increasing day by day for the discovery of therapeutic and nutraceutical agents from them and due to the versatile applications of those beneficial agents. The present study was thus designed to investigate the antioxidant and cytotoxic properties of methanolic *Averrhoa carambola* L. fruit extract along with the identification of the phytochemicals present in the extract. The antioxidant activities were determined by several assays: determination of total phenol content, assessing total flavonoids content, total antioxidant capacity assay and DPPH free radical scavenging assay. The *in-vitro* cytotoxic activity of fruit extract of *Averrhoacarambola* L. was determined by Brine Shrimp Lethality Biossay (BSLA). Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolics, carbohydrate, saponin, tannin in the methanolic extract of *Averrhoa carambola* L. fruit which was indicative of possible antioxidant and cytotoxic activity. The extract showed good antioxidant activities having total phenol content of 21.316±0.372 mg GAE/gm, total flavonoid content of 23.058±2.059 mg QE/gm, total antioxidant capacity of the extract was 152.368±2.791 mg AAE/gm. From DPPH free radical scavenging assay, the IC_{50} value of the extract was found 256.539µg/mL. From BSLA, $LC_{50}$was calculated as 243.571µg/mL. Our results suggest that methanolic extract of fruits of *Averrhoa carambola* L. possesses appreciable antioxidant and cytotoxic properties. Fruits of *Averrhoa carambola* L. may be a good source for natural antioxidants and a possible pharmaceutical supplement. Further studies are needed to isolate the responsible compounds and find out their mechanism of activity.

INTRODUCTION: Plants are considered as the prime source of many of the active ingredients of modern medicines. Medicinal Plants show therapeutic properties and exert beneficial pharmacological effects on the animal body [1].These plants contain substances that may act as precursors for synthesis of useful drugs[2].

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In recent years, traditionally used medicinal plants are gaining in popularity as alternative to the currently practiced medicine. Natural compounds have diversified structures that provide strategic starting point to synthesize new compounds [3, 4]. Natural products have historically played a significant role in new pharmaceuticals' discovery by becoming source of new drug candidates[5].

Averrhoa carambola L., commonly known as star fruit, is a fruit of the slow growing evergreen tree that belongs to Oxalidaceae family. It is locally known as "Kamranga", in Bangladesh. The herb is commonly grown in Malaysia, Taiwan, Thailand, Israel, Florida, Brazil, Philippines, China,

Australia, Indonesia, in the warmer parts of India, Bangladesh and other areas of the world with similar climate [1].The fruits are green when small and unripe but turn to yellow or orange when it is matured and ripe. The flesh is light yellow to yellow, translucent and very juicy without fiber. The fruit is used in fever, to relieve eye afflictions, as diuretic, to increase the salivary secretion, is considered as digestive, tonic and causes biliousness, as cooling medicine, possesses antiscorbutic properties, and as astringent and antioxidant activity. The fruit is also used for the treatment of throat inflammation, mouth ulcers, toothache, cough, asthma, hiccups, tight feeling in chest, nausea, vomiting, indigestion, food poisoning, colic, diarrhoea, jaundice, malarial splenomegaly and as cites [6].

We, now realize that many of the diseases are due to the oxidative stress (OS) that arise from the imbalance between formation and neutralization of prooxidants. Free radicals such as hydroxyl, peroxyl and superoxide radicals stabilize through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation, which causes OS. This damage is potential contributor to the pathogenesis of diseases like cancer, diabetes, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases [7]. Human body combats the toxic effects of the ROS (reactive oxygen species) regularly by a number of endogenous defense and protective mechanisms which include various enzymes and non-enzymatic antioxidants. Antioxidative compounds taken as foods, cosmetics and herbal medicine strengthen this self-defense mechanism.[8]

The aforementioned possibilities and understandings have recently lead to a great increase in interest in natural antioxidant of plant origin. Further, their promise in therapeutic agents for tackling free radicals and their use as nutraceuticals exerted great impact on the status of human health and disease prevention[8].

The current study was designed to screen phytochemicals and to investigate the antioxidant activity as well as brine shrimp lethality of methanolic extract of *Averrhoa carambola* L. fruit.

MATERIALS AND METHODS:

Study Design:

The present protocol was designed to screen the phytochemical group of compounds, antioxidant, and cytotoxic potentiality of fruit extract of *Averrhoa carambola* L. Methanol was used as a solvent for the extraction of the *Averrhoa carambola* L. fruit.

Collection of Plant Material:

The fruit of the plant was collected from Sirajgonj, Bangladesh and identified by the taxonomist of the Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh.

Preparation of Plant material & Extraction procedure:

Fruits of the plant were first washed with water to remove adhering dirt and then cut into small pieces and sun-dried for few days and then dried in a hot air oven (Size 1, Gallenkamp) at reduced temperature (not more than 50°C). Dried fruits were grinded into coarse powder using high capacity grinding mill. The powdered fruits were used for extraction by Soxhlet apparatus at elevated temperature (65°C) using methanol (500 mL). Extraction was considered to be completed when the fruit materials become exhausted of their constituents that were confirmed from cycles of colorless liquid siphoning in the Soxhlet apparatus. The filtrates obtained were dried at temperature of 40±2°C to have gummy concentrate of the crude extract. The extract was kept in a suitable container with proper labeling and then stored in cold and dry place for further use [9].

Phytochemical Screening Test:

The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents i.e. carbohydrates (through molisch's test and fehling's test), flavonoids, glucosides (through general test for glycoside and glucoside), steroids (through liebermann-burchard's test), saponins (through frothing test), tannins (through lead acetate test), alkaloids (through mayer's test, hager's test, wagner test and dragendorff's test). These phytochemicals were identified from their respective characteristic color changes as stated in the standard procedures [1].

Antioxidant Activity Evaluation:

Total Phenol Content Determination:

Total phenolic content of the prepared methanolic extract was determined using Folin–Ciocalteu Reagent (FCR) [9, 1]. One (1.0) mL of fruit extract (200µg/mL) was taken in a test tube and the standard (gallic acid) of different concentrations (5, 25, 50, 100 and 200 µg/mL) were taken in other five test tubes. All test tubes were marked accordingly. Five (5) mL of Folin–Ciocalteu reagent solution (diluted to 10 fold) were taken in the test tubes followed by the addition of 4 mL of 7.5% sodium carbonate solution in each. The test tubes were incubated at 20°C (30 minutes for standard solutions, and 1 hour for extract solution). Absorbance at 765 nm was measure during a UV-Vis spectrophotometer (Shimadzu UV PC-1600) against a blank. Total phenol contents of the fractions were expressed as Gallic acid equivalents (GAE) [10].

Determination of Total Flavonoids Content:

Total Flavonoid was determined using the Aluminum chloride colorimetric method described by Wang and Jiao [11]. One (1.0) mL of fruit extract (200µg/mL) and standard (Quercetin) of five different concentrations (5, 25, 50, 100 and 200 µg/mL) were taken in different marked test tubes. Then 3 mL of methanol was added to each of the test tubes followed by 200 µL of 10% aluminium chloride solution and 200 µL of 1 M potassium acetate solution. Finally, 5.6 mL of distilled water was added to the test tubes. After this the test tubes were incubated for 30 minutes at room temperature to complete the reaction. Absorbance of the solution was measured at 415 nm using a UV-Vis spectrophotometer (Shimadzu UV PC-1600) against a blank. Total Flavonoid content of the extract was expressed as Quercetin equivalents (QE).

Determination of Total Antioxidant Capacity:

Total antioxidant capacity of the fruit extract was determined by following the method described by ascorbic acid equivalents (AAE) [12]. Three hundred micro-liters (300µL) of extract (200µg/mL) and standard (ascorbic acid) of different concentrations (5, 25, 50, 100 and 200 µg/mL) were taken in different marked test tubes and then 3 mL of reagent solution (a mixture of 3.3mL of concentrated 98 % H_2SO_4, 0.381gm sodium phosphate and 0.494gm of ammonium molybdate prepared in a 100mL volumetric flask adjusting the volume to 100 mL with distilled water) was added to each test tubes. The setest tubes were then incubated at 95°C for 90 minutes to complete the reaction. Absorbance of each of the incubated solutions, after cooling to room temperature, was measured at 695 nm using a UV-Vis spectrophotometer (Shimadzu UV PC-1600) against a blank. Total antioxidant capacity of the extract was expressed as ascorbic acid equivalent (AAE).

DPPH Free Radical Scavenging Assay:

DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging activity of the plant extract was determined following the method described by Braca *et al*.[13]. One (1.0) mL fruit extract of different concentrations (6.25, 12.5, 25, 50, 100, 200, 500 µg/mL) and 1.0 mL standard (ascorbic acid) of different concentrations (2.5, 5, 10, 20, 40, 80 µg/mL) were taken in different pre-marked test tubes. Then, 2 mL of 0.004% methanolic DPPH solution was added to each test tube. All the prepared test tubes with their contents were then incubated for 30 minutes at room temperature. Absorbance of each of the incubated solutions was determined at 517 nm and IC_{50} value was calculated from the curve. (**Fig. 4**)

Brine Shrimp Lethality Bioassay (BSLA):

Lethal activity of the fruit extract was determined by Brine shrimp lethality bioassay described by Meyer *et al*.[14]. Brine shrimp lethality bioassay is a rapid, inexpensive and comprehensive bioassay and had let to the discovery of bioactive compounds of natural origin[14, 15]. Brine shrimp eggs (*Artemiasalina* leach) were hatched in simulated seawater with constant oxygen supply for two days, to get nauplii.

Stock solution of the sample was prepared by dissolving required amount of extract (20 mg) in specific volume (400µL) of pure dimethylsulfoxide (DMSO) and adding sea water to make the total volume 20 mL. At this point the stock solution gained concentration of the extract as 1µg/µL. Then specific volumes of stock solution was transferred into different test tubes so that the final

concentration of the extract becomes 1, 5, 10, 20, 50, 100,200 and 500µg/mL in the respective test tubes after volume adjustment to 5 mL with sea water. In the control tubes 75µL and 150µL DMSO were taken and volume was adjusted to 5mL with sea water (as in the sample tubes). Vincristine sulfate was used as positive control and evaluated at very low concentration (10, 5, 1, 0.5, 0.25, 0.125 and 0.06µg/mL). With the help of a Pasteur pipette 10 living nauplii were put to each of the test tubes. After 24 hours the test tubes were observed and the number of nauplii survived in each test tube was counted. The mortality was corrected using Abott's formula [16].

$$P_t = [(P_o - P_c)/(100 - P_c)] \times 100$$

Where, P_t= Corrected mortality, P_o= Observed mortality and P_c= Control mortality.

LC_{50} values of the test samples after 24 hours are obtained by regression analysis.

RESULTS:

Phytochemical Screen Test:

In the present study, various qualitative tests were done to detect the presence of different phytochemical compounds in the methanolic extract of the fruits of *Averrhoa carambola* L. Preliminary phytochemical screening of the crude extract of the fruit of *Averrhoa carambola* L. showed the presence of different kind of phytochemical groups. The obtained results are summarized in the **Table 1**.

TABLE 1: RESULTS OF PHYTOCHEMICAL GROUP TESTED WITH THE FRUIT EXTRACT (METHANOLIC) OF *AVERRHOACARAMBOLA* L.

Phytochemical Group of Compounds	Results
Alkaloid	+
Carbohydrate	+
Flavonoid	+
Glucoside	–
Glycoside	–
Saponin	+
Steroid	–
Tannin	+

'+' sign indicates presence of phytochemical group of compounds while the '-' sign indicates absence of phytochemical group of compounds tested for

Antioxidant Activity Evaluation

Total Phenol Content Determination: The total phenolic compounds content of the test solutions were calculated using the standard line (**Fig. 1**)of Gallic acid (y = 0.0097x + 0.0558, r^2 = 0.9985)**.** The results were expressed as gallic acid equivalents (GAE) per gram of the extract. The extract was found to have total phenol content 21.316±0.372 mg GAE/gm of the extract (**Table 2**).

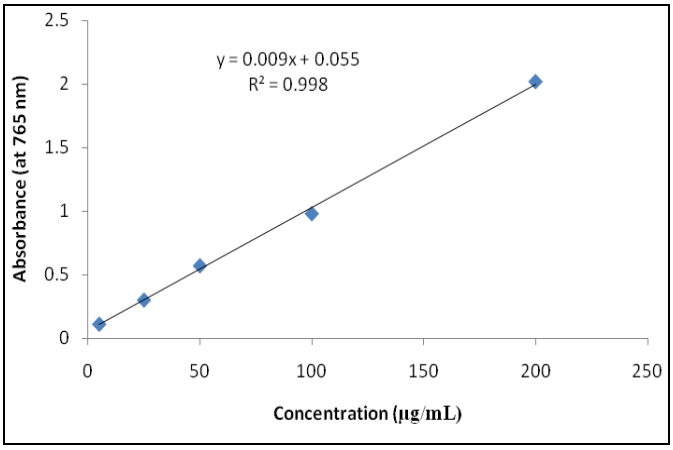

FIG.1: CALIBRATION LINE OF GALLIC ACID STANDARD

Total Flavonoid Content Determination:

The total Flavonoid content of the extract was calculated using the standard line (**Fig. 2**) of Quercetin (y=0.0103x-0.0115, r^2 = 0.9988). Flavonoid content of the extract was expressed as mg/gm Quercetin equivalent (QE). The extract was found to have total flavonoid content 23.058±2.059mg QE/gm of the extract (**Table 2**).

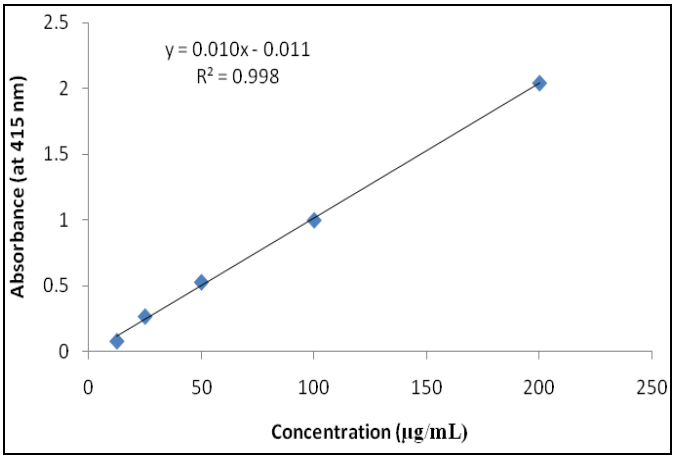

FIG.2: CALIBRATION LINE OF QUERCETIN STANDARD

Total Antioxidant Capacity Assessment:

Total antioxidant capacity of *Averrhoa carambola* L. fruit extract was evaluated by the phosphomolybdenum method and was expressed as ascorbic acid equivalents (AAE) per gram of fruit extract. Total antioxidant capacity of the test

samples was calculated using the standard line (**Fig. 3**) of ascorbic acid (y=0.0032x-0.0299, r^2 = 0.9837). Methanolic extract of the fruit of *Averrhoa carambola* L. was found to contain total antioxidant capacity of 152.368±2.791mg AAE/gm of the extract (**Table 2**).

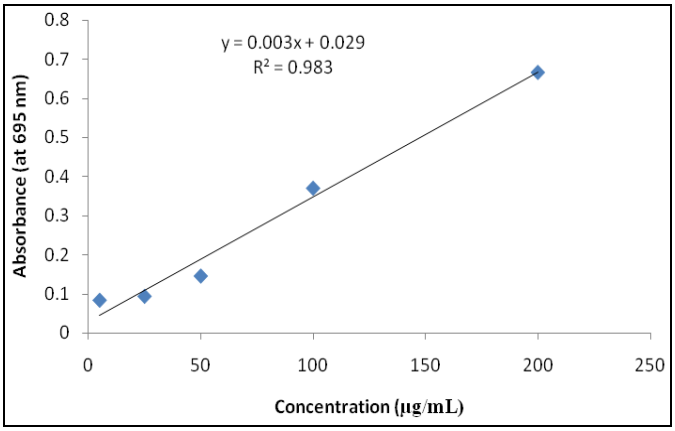

FIG.3: CALIBRATION LINE OF ASCORBIC ACID STANDARD

TABLE 2: SUMMARIZED RESULTS OF ANTIOXIDANT CAPACITY OF THE FRUIT EXTRACT OF *AVERRHOACARAMBOLA* L. IN TERMS OF EQUIVALENT OF DIFFERENT STANDARDS

Total phenol (mg GAE/gm of extract)	Total Flavonoid (mg QE/gm of extract)	Total antioxidant (mg AAE/gm of extract)
21.316±0.372	23.058±2.059	152.368±2.791

DPPH Free Radical Scavenging Assay:

DPPH scavenging assay has been widely used to evaluate the free radical scavenging capacity of antioxidants. The IC_{50} value of the extract of *Averrhoa carambola* L. is shown in the **Table 3**. Methanolic extract of *Averrhoa carambola* L. fruit was found to have the IC_{50} value of 256.539 µg/mL. The standard antioxidant, ascorbic acid, showed IC_{50} value of 16.880 µg/mL (**Table 3**). **Fig. 4** shows the concentration dependent free radical scavenging activity of the extract and standard.

Brine Shrimp Lethality Bioassay (BSLA):

LC_{50} values for the extract as well as for the standard were calculated as stated in the experimental section under BSLA and the obtained results are depicted in Table 3.In this study, methanolic extract of fruit of *Averrhoa carambola* L. showed toxicity to brine shrimp nauplii, with LC_{50} value of 243.571 µg/mL whereas anticancer drug Vincristine sulphate showed LC_{50} value of 2.48µg/mL (**Table 3**).

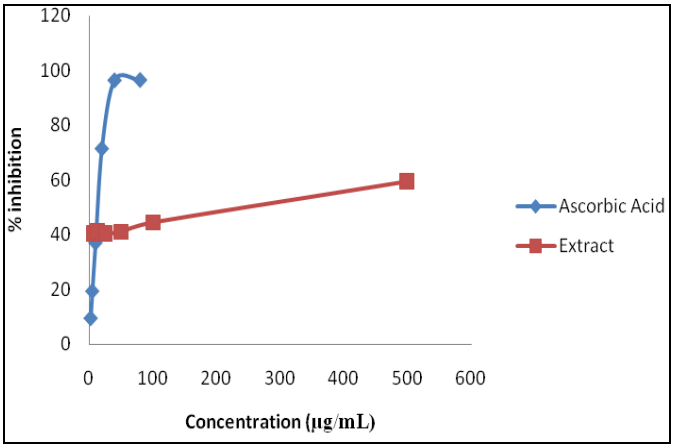

FIG.4: DPPH RADICAL SCAVENGING ACTIVITY OF THE EXTRACT AND THE STANDARD ASCORBIC ACID

TABLE 3: IC_{50} AND LC_{50} VALUES OF THE METHANOLIC EXTRACT OF *AVERRHOACARAMBOLA* L. FRUIT

IC_{50}(from DPPH Scavenging Assay) in µg/mL		LC_{50} (from Brine Shrimp Lethality Bioassay) in µg/mL	
Extract	Ascorbic Acid	Extract	Vincristine sulphate
256.539	16.880	243.571	2.48

DISCUSSION: Phytochemical screening of the methanolic extract of fruit of *Averrhoa carambola* L. confirmed the presence of alkaloid, flavonoid, carbohydrate, tannin and saponin in the extract (**Table 1**). There are studies, where it was reported that fruit of *Averrhoa carambola* L. is rich in antioxidants, especially polyphenol, flavonoids (proanthocyanidins, epicatechin) and vitamin C, carbohydrates [6]. Findings of the present study is thus in line with the results of other works.

Tannins possess anti-inflammatory and anticancer activity[17]; flavonoids are antimicrobial, antiviral, antiatherosclerosis, cardioprotective, antiulcerogenic, cytotoxic, antineoplastic, mutagenic, antidiabetic, anti-inflammatory, antioxidant, anti-aging, antihepatotoxic, antihypertensive, hypolipidaemic and antiplatelet agents[18]; alkaloids possess anticancer activity [19] and saponins are antimicrobial agent and maintain the blood cholesterol level [17]. Presence of these compounds in *Averrhoacarambola* L. fruit extract accounts the antioxidant and cytotoxic effect of the extract.

Fruit extract of *Averrhoa carambola* L. showed moderate amount of phenolic compounds content (21.316±0.372 mg GAE/gm of the extract) (**Table 2**). Results of the present study is comparable to other reported findings such as assaying with freeze-dried fruit residue it was found that total polyphenolic compounds content of 33.2 ± 3.6 mg GAE/gm of freeze-dried fruit residue [20]. Many investigations revealed that phenolics and flavonoids content contribute to the antioxidant activities of plant materials [21, 22].

It was revealed from the present study that the fruit extract of *Averrhoa carambola* L. possess moderate amount of flavonoids (23.058±2.059 mg QE/gm of the extract (**Table 2**), which is slightly lower than previously reported 34.26±1.73 mg QE/gm of extract[23]. This discrepancy might be due to the use of water extract of the fruit in that study. Flavonoids show notable antioxidant capacity. This may arise from several mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for free radical generation [24]. Depending on their structure, flavonoids are able to scavenge practically all known ROS [25].

The total antioxidant activity of fruit extract of *Averrhoa carambola* L. was evaluated in the present study from its ability to reduce Phosphate/Mo(VI) complex to Phosphate/Mo(V). According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species [26]. *Averrhoa carambola* L. fruit extract showed significant total antioxidant capacity152.368±2.791 mg AAE/gm of the extract (**Table 2**). High level of total antioxidant capacity of *Averrhoa carambola* L. Fruit was reported previously [20] and that justify the finding of the present study.

DPPH radical scavenging is a popular and a reliable method for screening the free radical scavenging activity of compounds or antioxidant capacity of extracts of plant parts[27, 28]. In the present study, DPPH radical scavenging assays with *Averrhoa carambola* L. fruit extract showed increasing scavenging effect to DPPH radicals in a similar manner to that of the reference antioxidant ascorbic acid (**Fig. 4**) and the activity of the extract was moderate (IC_{50} = 256.539 µg/mL, **Table 3**). This free radical scavenging activity might be due to the presence of phenols and flavonoids in the extract. *n*-Butanol extract of the fruit of *Averrhoa carambola* L.was reported earlier to have IC_{50} value of 124.48 µg/mL in DPPH scavenging assay [29], which ismuch less than that of the finding (IC_{50} = 256.539 µg/mL, **Table 3**) of the present study. This difference might be due to various extrinsic factors such as cultivation, environmental, different source of plant materials (here, fruit) etc. And it could be also for the use of less polar *n*-butanol solvent for extraction in the reported study.

Brine Shrimp lethality bioassay, after 24 hours, with the fruit extract of *Averrhoa carambola* L. showed moderate lethality (LC_{50} = 243.571 µg/mL, **Table 3**) and this result indicating its cytotoxic potentiality. $LC_{50}\leq$ 1000µg/mL demonstrates presence of bioactive compound [14] and $LC_{50}\leq$ 500 is deemed to be toxic to brine shrimp [30]. In an earlier study, very low LC_{50}value was reported (800µg/mL) [29], where the extract of the fruit of *Averrhoa carambola* L. was prepared using *n*-hexane. On the other hand, very high cytotoxicity (LC_{50} = 19.95 µg/mL) was reported for the ethanol extract of stem bark of the *Averrhoa carambola* L. plant[31]. These facts suggest that difference in cytotoxic potential of various extracts may vary depending on the parts of plants used as well as solvents used for extraction. However, the observed cytotoxic action of the extract of the present study could be accounted for the presence of compounds like alkaloids, phenolics and flavonoidsas these compounds show anticarcinogenic property [32].

CONCLUSION: From the present study, it was observed that the methanolic extract of *Averrhoa carambola* L. fruit possesses good antioxidant potential and it could be rationalized taking into account the presence of phenolics and flavonoid contents in it. The extract also showed moderate cytotoxic activity in BSLA, which might be attributed to the presence of alkaloid and flavonoid in the extract. It is advantageous to use the plant antioxidants in therapeutic drugs for the implications of human health as the antioxidants from natural products greatly contribute to the prevention of pathological disorders caused by free

radicals and since the antioxidants of plant sources are safer and cheaper than the synthetically produced. However, further studies are needed to demonstrate *in-vivo* antioxidant capacity of the studied fruit as well as to identify and isolate the responsible compounds, which may lead to new drug discovery.

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REFERENCES:

1. Ghani A: Medicinal Plants of Bangladesh. The Asiatic Society of Bangladesh, Dhaka, Bangladesh. Second Edition. 2003.
2. Hossain MS, Chowdhury MEH, Das S, Chowdhury IU. In-Vitro Thrombolytic and Anti-inflammatory Activity of Swertia chirata Ethanolic Extract. Journal of Pharmacognosy and Phytochemistry 2012; 1(4): 99-104.
3. Clardy J, Walsh C: Lessons from natural molecules. Nature 2008; 432 (7019): 829-837.
4. Koehn FE, Carter GT: The evolving role of natural products in drug discovery. Nature Review Drug Discovery 2005; 4:206–220.
5. Dias DA, Urban S, Roessner U: A Historical Overview of Natural Products in Drug Discovery. Metabolites 2012; 2(2): 303–336.
6. Payal G, Pankti K, Chakraborty M, Jagadish VK: Phytochemical and pharmacological profile of averrhoacarambolalinn: an overview. International Research Journal of Pharmacy 2012; 3 (1): 88-92.
7. Islam S, Nasrin S, Khan MA, Hossain ASMS, Islam F,Khandokhar P, Mollah MNH, Rashid M, Sadik G, RahmanMAA, Alam AHMK: Evaluation of antioxidant and anticancer properties of the seed extracts of SyzygiumfruticosumRoxb. growing in Rajshahi, Bangladesh. BMC Complement Altern Med 2013; 13:142.
8. Chaabane F, Boubaker J, Loussaif A, Neffati A, Kilani-Jaziri S, Ghedira K, Chekir-Ghedira L. Antioxidant, genotoxic and antigenotoxic activities of daphne gnidium leaf extracts. BMC Complementary and Alternative Medicine. 2012; 12: 153.
9. Cannel RJP: How to approach the isolation of a natural product. In: Natural products isolation. New Jersey: Humana Press. First Edition 1998; 1-51.
10. Velioglu YS, Mazza G, Gao L, Oomah BD: Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agricultural and Food Chemistry 1998; 46:4113–17.
11. Wang SY, Jiao H: Correlation of antioxidant capacities to oxygen radical scavenging enzyme activities in blackberry. Journal of Agricultural and Food Chemistry 2000; 48:5672-76.
12. Prieto P, Pineda M, Aguilar M: Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochemistry 1999; 269:337–341.
13. Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I: Antioxidant principles from Bauhinia terapotensis. Journal of Natural Products 2001; 64: 892-895. 13
14. Meer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE : Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. Planta Medica 1982; 45(5): 31-34.
15. Musa AA: Cytotoxicity Activity and Phytochemical Screening of Cochlospermum tinctorium Perr Ex A. Rich Rhizome. Journal of Applied Pharmaceutical Science 2012; 2(7): 155 – 159.
16. Abbott WS: A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 1925; 18: 265-267.
17. Ahmad B, Khan MR, Shah NA, Khan RA: In vitro antioxidant potential of dicliptera roxburghiana. BMC Complementary & Alternative Medicine 2013; 13: 140.
18. Rathee P, Chaudhary H, Rathee S, Rathee D, Kumar, Kohli K: Mechanism of Action of Flavonoids as Anti-inflammatory Agents: A Review. Inflammation & Allergy - Drug Targets 2009; 8: 229-235.
19. Pan L, Chai HB, Kinghorn AD: Discovery of new anticancer agents from higher plants. Front Biosci (Schol Ed) 2013; 4: 142–156.
20. Shui G, Leong LP: Residue from star fruit as valuable source for functional food ingredients and antioxidant nutraceuticals. Food Chem 2006; 97: 277 - 284.
21. Ravipati AS, Zhang L,Koyyalamudi SR, Jeong SC, Reddy N, Bartlett J,Smith PT,Shanmugam K, Münch G, Wu MJ, Satyanarayanan M, Vysetti B: Antioxidant and anti-inflammatory activities of selected Chinese medicinal plants and their relation with antioxidant content. BMC Complement Altern Med 2012; 12:173.
22. Oskoueian E, Abdullah N, Saad WZ, Omar AR, Ahmad S, Kuan WB, Zolkifli NA, Hendra R, Ho YW: Antioxidant, anti-inflammatory and anticancer activities of methanolic extracts from Jatropha curcas Linn. Journal of Medicinal Plants Research 2011; 5(1), 49-57.
23. Asna AN, Noriham A: Antioxidant activity and bioactive components of oxalidaceae fruit extracts. Malaysian J Analyt Sci 2014; 18 (1): 116-126.
24. Benavente-Garcia O, Castillo J, Marin FR, Ortuno A, Del Rio JA: Uses and properties of Citrus flavonoids. J Agric Food Chem 1997; 45: 4505–4515.
25. Mitra K, Uddin N: Total phenolics, flavonoids, proanthrocyanidins, ascorbic acid content and in vitro antioxidant activities of newly developed isolated soya protein. Discourse J of Agri Food Sci 2014; 2(5): 160-168.
26. Birasuren B, Kim NY, Jeon HL, Kim MR: Evaluation of the Antioxidant Capacity and Phenolic Content of Agriophyllum pungens Seed Extracts from Mongolia. Preventive Nutrition and Food Science 2013; 18(3): 188-195.
27. Mazumdar MEH, Rahman S: Pharmacological evaluation of Bangladeshi medicinal plants for antioxidant activity. Pharmaceutical Biology 2008; 46 (10– 11): 704–709.
28. Espín JC, Soler-Rivas C, Wichers HJ: Characterization of the total free scavenger capacity ofvegetable oils and oil fractions using 2, 2 diphenyl-1-picrylhydrazyl radical. Journal of Agricultural and Food Chemistry 2000; 48: 648-656.
29. Saghir S, Sadikun A, Khaw KY, Murugaiyah V: Star fruit (Averrhoa carambola L.): From traditional uses to

pharmacological activities .Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 2013; 12(3): 209 – 219.

30. Déciga-Campos M, Rivero-Cruz I, Arriaga-Alba M, Castañeda-Corral G, AngelesLópez GE, Navarrete A: Acute toxicity and mutagenic activity of Mexican plants used in traditional medicine. Journal of Ethnopharmacology 2007; 110: 334-42.
31. Das J, Datta Z, Saha A, Nur SM, Barua P, Rahman MM, Chowdhury KAA, Chowdhury MM, Chowdhury RH, Mannan A: A comprehensive study on antioxidant, antibacterial, cytotoxic and phytochemical properties of Averrhoa carambola. International Journal of Bioassays 2013; 02 (05): 803-807.
32. Abliz G, Mijit F, Hua L, Abdixkur G, Ablimit T, Amat N, Upur H: Anti-carcinogenic effects of the phenolic-rich extract from abnormal Savda Munziq in association with its cytotoxicity, apoptosis-inducing properties and telomerase activity in human cervical cancer cells (SiHa). BMC Complementary and Alternative Medicine 2015; 15:23.

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