

# In vitro Antibacterial and Antioxidant Activities of Star Fruit (*Averrhoa carambola*)

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**Abstract**—The present study, the potential antibacterial and antioxidant activities of *Averrhoa carambola* fruits were evaluated in terms of inhibition zone diameter and total reducing power assay. All fruit extracts were higher inhibition zone than juice. Fruit extract inhibited human pathogens; 21mm inhibition on *Salmonella typhi*, 20 mm on *Escherichia coli*, 19 mm on *Shigella boydii* than ampicillin. The highest inhibition zones of fruit extracts against on *Salmonella typhi*, and *Staphylococcus aureus* were 21 mm each. The presence of antioxidant activity was preliminary tested by phosphomolybdic acid. The two portions; juice and extract of samples were estimated by phosphomolybdenum revealed potent antioxidant activities.

**Keywords** —antibacterial, antioxidant, phosphomolybdate, dot blot, *Averrhoa carambola*.

## I. INTRODUCTION

The importance of fruits and vegetables in our diet is increasingly growing as one of the main sources of antioxidants and reported to be beneficial to age related diseases, cancers, inflammation, heart disease and acceleration of the ageing process [1-5]. Star fruit (*Averrhoa carambola* L.) belongs to the Oxalidaceae family is a tree fruit commonly known as carambola and zaung yar in Myanmar. The edible star fruit is available locally in various parts of Myanmar and predominantly eaten fresh as a vegetable and processed to various products like pickles, salad, jam and dried fruit. The powdered seed concoction of the fruit is traditionally used for its medicinal properties to treat hemorrhoids, fever, eczema, diarrhea and asthma [6]. Extensive reports on the strong free radical scavenging potential of juice and residue of star fruit cultivated in Singapore and Indonesia have attributed its capacity to the rich procyanidin polymers and  $\beta$ - carotene [7, 8]. It is a very rich source of vitamins and also serves as a good source of phytochemicals. The objective of the study was to analyze the capacities of antibacterial and reduction power assay.

## II. MATERIALS AND METHODS

### Preparation of star Fruit Sample

Fresh star fruits were collected from Sinphyukyun, Magway Region, Myanmar. Samples were prepared to juice and extract. 500 gram of Fruits were cut to slices and 60 °C oven dried until moisture were got off. The dried slices were grinded to powder with blender and the powder were macerated with 70% ethanol for one month. 100 gram Fruits were pressed to form juice filtered and placed to 4 °C to 7 °C in refrigerator before experiment.

### Test Organisms

The six test organisms were provided by Department of Biotechnology, Mandalay Technological University. The bacteria used in study were *Bacillus cereus*, *Salmonella typhi*, *Escherichia coli*, *Shigella boydii*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*.

### In vitro Antibacterial Assay

Antibacterial activities of star fruits juice and extracts were tested against a total of six bacteria [9, 10]. (Bennet et al., 1966 and Janssen et al., 1987). Bacterial stock cultures were inoculated to nutrient broth and incubated at 37°C for 6 to 12 hours. The nutrient medium was prepared on the six plates. The bacterial broth was streaked on the surface of each plate using sterile cotton. Sterilized 8 mm cork borer was used to make wells and 50  $\mu$ l of juice and 30mg/ml extract were placed into each well. Ethanol(70 %) was used as control and 25 $\mu$ g/ml ampicillin was used as positive control. After overnight culture incubation, zone of inhibition was measured by 1 mm accuracy scale ruler. The determination of antibacterial activity was carried out in triplicates.

### Screening on Total Antioxidant Activity of Star Fruit Extract

Total antioxidant activity of star fruit extracts was done to screen the presence of antioxidant. Aliquots of 5 $\mu$ l (of a 10 mg/ ml final solution) of each extract were applied on Merck Silica gel F254 plates and allowed to dry for a few minutes. Drops of each sample and positive control were placed in two rows. The sequence was according to decreasing quantity: 2000  $\mu$ g, 1000  $\mu$ g, 500  $\mu$ g, 250  $\mu$ g and 125  $\mu$ g extracts and 500  $\mu$ g, 250  $\mu$ g and 125  $\mu$ g, 62.5  $\mu$ g and 31.25  $\mu$ g ascorbic acid. The staining of TLC plates was done according to the method of Takao et al. (1994) with modifications [11]. The spray reagent (20% phosphomolybdic acid) was sprayed on the spots [12,13]. The color development was checked by the presence of antioxidant.

**Total antioxidant activity by phosphomolybdate**

Total antioxidant activities of juice and extract were estimated by phosphomolybdenum assay [14].

**Procedure**

Ethanol extracts (from 2000 mg to 125mg/ml in ethanol) and juice (from 500 ul/ ml to 31.125 ul/ml in distilled water ) of starfruits in different concentrations were prepared and 0.5 ml were added to each test tube individually containing 5 ml of Molybdate reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate ) solution. The blank was used as ethanol instead of sample. These tubes were kept at 95 °C for 90 min. After incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695 nm. Mean values from three independent samples were calculated for each extract. Ascorbic acid was used as positive reference standard.

**III. RESULTS AND DISCUSSION****Antibacterial activity of *Averrhoa carambola* fruit juice and extract**

Antibacterial activity of *Averrhoa carambola* fruit juice and extracts were tested against six two gram positive bacteria and four negative bacteria. Ethanol (70%) was used as negative control and 25 ug ampicillin was positive control. Inhibition zone diameter of activities were expressed in mm and showed in fig.1 and table 1.

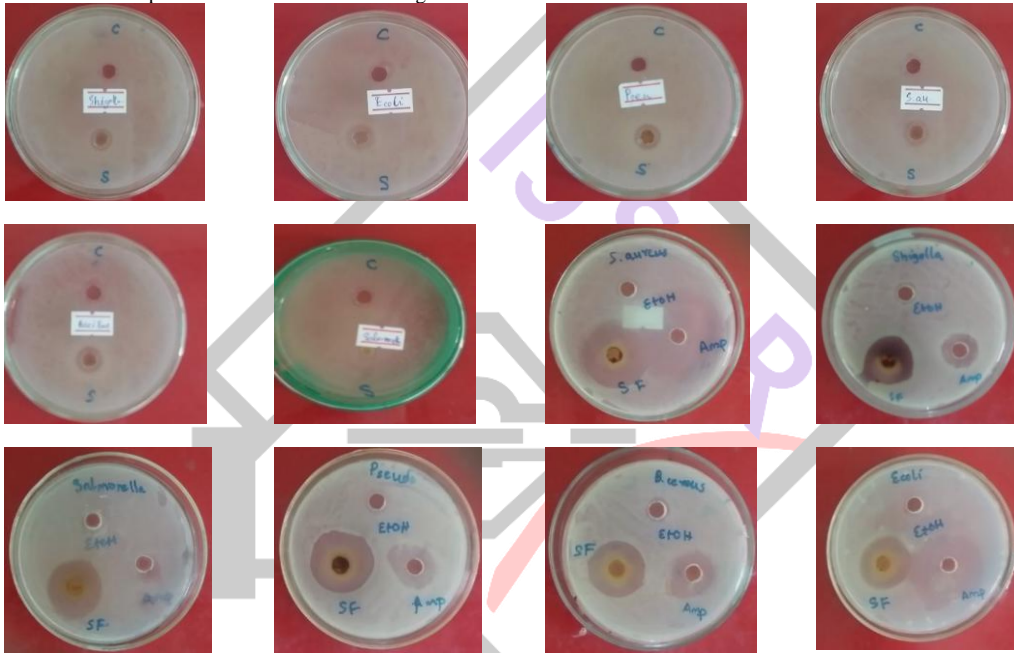


Figure1. Antibacterial Activity of Star Fruit Juice and Extract on Nutrient Agar Against Six Bacteria

Table 1. Inhibition Zone Diameter of Star Fruit Samples against Bacteria

Microorganisms	SF extract(sf) mm	SF juice(s)mm	Control (c/EtOH) mm	Ampicillin (amp) mm
<i>Bacillus Cereus</i>	15±2.	3±1.00	0	23±2.2
<i>Pseudomonas aeruginosa</i>	15±1.	2±0.80	0	24±2.5
<i>Salmonella typhi</i>	21±3.	2±0.70	0	0
<i>Shigella boydii</i>	19±2.	2±0.50	0	12±2.1

Comment [T1]:

<i>Staphylococcus aureus</i>	21±3.	3±0.80	0	34±3.4
<i>Escherichia coli</i>	20±2.	3±0.10	0	17±2.9

According to the result, star fruit extract gave the more antibacterial activity than juice. *Salmonella typhi*, *Escherichia coli*, *Shigella boydii* were more sensitive to star fruit extract compared to ampicillin. The extract was high activity on all six bacteria. Among them, the most susceptibilities were 21 mm inhibition zone of *Salmonella typhi*, and *Staphylococcus aureus*.

#### Screening of Total antioxidant assay by phosphomolybdic acid

Difference concentration of ethanol extract of star fruit showed the presence of antioxidant activity. The dot-blot assay on TLC plate showed colored spots where the aliquots of different concentrations of each extract and L- ascorbic acid (Vitamin-C) were dropped on plate according to the method of the Takao et al. (1994) with modification. The blue area on the plate indicates reducing power activity or antioxidant activity. The more intense the blue colour, the greater the antioxidant activity is as shown in Fig.2.

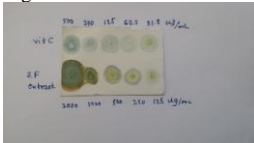


Figure 2. Screening of Antioxidant of Star Fruit Extract by Dot Blot Assay on TLC Plate

#### Phosphomolybdic Assay

The total antioxidant potential (TAP) of star fruit extract has been evaluated by phosphomolybdic assay (Prieto et al, 1999) and was measured in UV spectrophotometer. The total antioxidant activity of juice, fruit extract and were showed compared with as ability of total antioxidant of ascorbic acid and shown in figure (3). Increased TAP is proportional to increased absorbance of reaction mixture (Morales & Paredes, 2014) [15]. The reduction rate of Mo (VI) to Mo (V) is evaluated by phosphomolybdic assay, thereby providing direct estimation of antioxidant reducing capacity. It is used to quantitatively assess the reduction reaction degree among antioxidant, oxidant and molybdenum ligand, by formation of green complex without the involvement of free metal ions (Phatak and Hendre, (2014) [16].

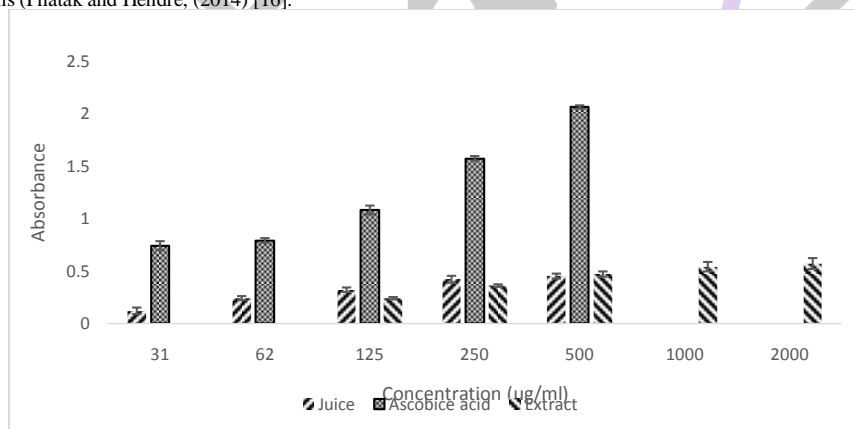


Figure 3. Total Antioxidant Potential (TAP) of Star Fruit Juice and Extract

#### IV. CONCLUSION

Present study concludes that *Averrhoa carambola* fruit juice and ethanolic extract possess antibacterial activity and antioxidant activity containing of vitamin C, vitamin A, and phytoconstituents such as saponins, alkaloids, flavanoids and tannins in extract. The active constituents alone or in combination may be responsible for these activities. Potential compound from ethanolic extract of the fruit of *Averrhoa carambola* should be isolated responsible for other activities besides antibacterial and antioxidant activities.

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