Investigation on some Chemical Constituents and Bioactivities of *Averrhoa Carambola* L. Fruit (Zaung -Yar)

Phyu Phyu Zaw[1], Saw Hla Myint[2], Sanda Moe[3]

Abstract

This research is concerned with the chemical investigation and some biological activity of Zaung-yar fruit (*Averrhoa carambola* L.).The antioxidant activity of *Averrhoa carambola* L. fruit was assessed by DPPH radical scavenging activity assay.The ethanolic extract of Zaung-yar fruit has significant antioxidant property (IC_{50}= 9.165 ppm) when compared to standard antioxidant, ascorbic acid (IC_{50}= 5.916 ppm). In antimicrobial activities of pet-ether,ethyl acetate,70% ethanol and water extracts were tested against six different microorganisms (*Bacillus subtilis, Bacillus pumalis, Candida albicans, Mycobacterium, Pseudomonas aeruginosa* and *Staphylococcus aureus*) by agar well diffusion methods. Ethyl acetate,70% ethanol and water extracts showed moderate activities on all tested organisms. Pet-ether extract was inactive. The preliminary phytochemical tests revealed the presence of phenolic compound, glycosides, tannis, sponins, flavonoids, terpenoids and organic acid. For isolation and characterization, six compounds were isolated from deacidified ethyl acetate extract of Zaung-yar fruit by column chromatography in silica gel with pet-ether:ethyl acetate (1:1.5) solvent system. The compound was characterized as abscisic alcohol by using UV, FT IR spectroscopic methods and by reference to literature. The other five still need to be identified.

Keywords: antioxidant, antimicrobial, abscisic alcohol, *Averrhoa carambola* L.

Introduction

Zaung-yar (*Averrhoa carambola* L.) fruit belongs to the botanical family Oxalidaceae. It is yellow or green in colour. Slices cut in cross-section are star shaped, which is why it's also called star fruit. Star fruit can be classified into two main types. The smaller one is (very sour type) and the larger one is (sweet type). Zaung-yar fruit is a good source of magnesium, potassium, phosphorous, β-carotene, fiber, vitamin C and low calorie. Because of high nutritional value, Zaung-yar fruit is commonly used in preparation of juice, pickles, salad, eat it raw and cooked with fish. The *Averrhoa carambola* L. plant is originated in tropical countries such as Myanmar, India, Malaysia, Indonesia, Philippine and is also distributed around the world. Zaung-yar is a slow growing short trunked evergreen tree and best grow up to 6 to 9 m in height. The flowers are small clusters of red or purple colour. Zaung-yar fruit can be medicinally useful for its antioxidant, antimicrobial, antipyretic, anti-inflammatory, antidiarrheal, diuretic properties and it also reduce fatty liver risk and cholesterol. However, people with kidney problems should not eat star fruit due to its high oxalate content (Muthu,N., 2016).

Botanical Description of Zaung-yar (*Averrhoa carambola* L.)

Family : Oxalidaceae
Genus : Averrhoa
Species : carambola
Botanical Name : *Averrhoa carambola* L.
English Name : Star fruit
Myanmar Name : Zaung-yar
Part of used : Fruit
(The Wealth of India 2006)

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1. Lecturer, Dr., Department of Chemistry, Dagon University
2. Part Time Professor, Dr., Department of Chemistry, Yangon University
3. Professor, Dr., Department of Chemistry, West Yangon University

Figure 1 Photograph of plant, flowers and star shaped fruit when cut horizontally of Zaung-yar (*Averrhoa carambola* L.)

Material and Methods

Isolation of Compounds from the Ethyl Acetate Extract of Zaung-yar Fruit

The air-dried powder of Zaung-yar fruit (300g) was defatted with 70% ethanol by percolation. The hydroalcoholic extract was filtered and concentrated in vacuum rotatory evaporator to give dark brown residue. This residue was extracted with pet-ether. Two layers were formed, the lower aqueous layer was then successively extracted with EtOAc. Finally, the upper EtOAc layer was deacidified with 5% $NaHCO_3$ after evaporation and obtained residues of deacidified EtOAc extract (1g). The deacidified extract (1 g) was fractionated on a silica gel column (60 x 2.5cm). Elution was carried out using PE : EtOAc (1:1.5) solvent mixture to give six compounds.

In Vitro Antioxidant Activity of Ethanolic Extract from Zaung-yar Fruit by DPPH Assay

Ethanolic extract extract (2 mg) was dissolved in 50% ethanol-water and made up the volume to 10 mL to get a stock solution at concentration of 200 μg/mL. By diluting this stock solution with 50% ethanol-water, different concentrations of test sample solutions (1.625,3.125,6.25,12.5,25,50,100 and 200 μg/mL) were prepared. DPPH powder (2.364 mg) was dissolved in 95% ethanol by the aid of vortex mixer and the volume was made up to 100 mL. This solution was freshly prepared and kept in brown color flask in refrigerator no longer than 24 hours. (Nay Mar Soe, 2005)

Control, blank and sample solution were also prepared. All these solutions were allowed to stand at room temperature for 30 minutes. Then, the absorbances was measured at λ 517 nm and recorded on spectrophotometer UV-160 1PC (P/N 206-6750), Shimadzu corporation. Absorbance of individual solution was measured in triplicate and % inhibition of each sample solution was calculated. Then, IC_{50} value was calculated by linear regressive excel program.

Determination of Antimicrobial Activity of Various Crude Extract of Zaung-yar Fruit

PE, EtOAC, 70%EtOH and water extracts of *Averrhoa carambola* L. fruit was prepared. For *in vitro* antimicrobial activity of crude extracts were studied by agar well diffusion method. The bacterial strains used in this antimicrobial assay were as follows: *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis, Candida albican* and *Mycobacterium* species (Cruckshank,1960).Theextracts (1g each) were introduced into sterile petridishes and dissolved in 1mL of their respective solvents of PE , EtOAc, 70% EtOH and H_2O. The diameter of the clear circular zone formed around the agar well against the cloudy background of microbial growth was measured. This is a semiquantitative

measure of the antimicrobial activity against the tested microorganism of a particular fruit extracts.

Results and Discussion

Characterization and Identification of the Isolated Compound no.6

UV Spectroscopy

In the UV spectrum of the isolated compound no.6, two bands can be observed with absorption maxima at 202 and 235 nm. After adding NaOH, the bands appeared at 205 and 235nm, showing hardly any bathochromic shift. This observation suggests the absence of phenolic group: the K band and at 235 nm is in accord with the presence of an enone strutrure.

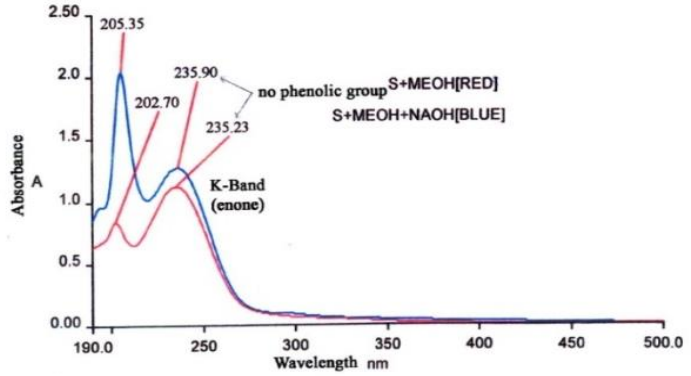
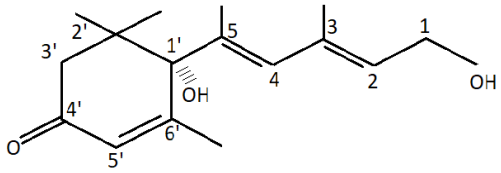


Table 1.

Compo	+NaOH)
Compo	

IR Spectroscopy

The FT IR spectrum of the isolated compound no.6 is shown in figure 3. In the FT IR spectrum of the isolated compound no.6 , the broad O-H stretching band at 3448 cm^{-1} indicates the presence of OH group in the isolated terpene. The usual C-H stretching of -CH_3, >CH_2 can also be observed at 2962 cm^{-1}. The carbonyl strectching band at 1664 cm^{-1} suggests an α , β unsaturated carbonyl structure. The symmetric in-plane bending band of CH_3 can be seen at 1375 cm^{-1}. The C-O stretching bands at 1161-1122 cm^{-1} and, 1072-1030 cm^{-1} may be assigned, respectively to 3˚ and 1˚ alcohols. The =C-H out of plane bending bands respectively, of trans disubstituted alkene and trisubstituted alkene and trisubstitued enone, can also 971 and 841-810 cm^{-1}.According to the literature (Lutz, 1994) among the known recorded compounds in the *Averrhoa carambola* L. fruit, the sesquiterpence, abscisic alcohol satisfies the above observation. The structure is



Abscisic alcohol

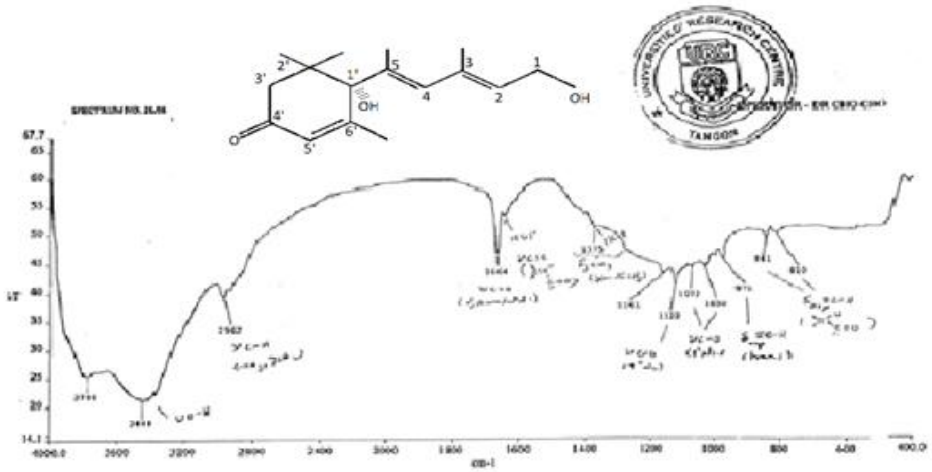


Figure 3. FTIR spectrum of isolated compound no.6

Table 2 . FT IR Band Assignment of Isolated Compound no.6

Wave number (cm^{-1})	Band Assignments
3448	O-H Stretching vibration
2962	Aliphatic C-H stretching vibration
1664	C=O stretching vibration
1645	C=C stretching vibration
1375	Aliphatic C-H in plane bending vibration
1161,1122	C-O stretching vibration of 1°alclhol
1072,1030	C-O stretching vibration of 3°alclhol
971,841,810	=CH out of plane bending vibration

In Vitro Antioxidant Activity of Ethanolic Extract of Zaung- yar (*Averrhoa carambola* L.) Fruit

The *in vitro* antioxidant activity of the ethanolic extract of Zaung-yar fruit was determined on the basis of their scavenging activity of the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical. Scavenging DPPH free radicals is a simple and acceptable method to evaluate the antioxidative activity of antioxidant. This method associates with change in the absorption, which can be followed spectroscopically.

Ethanolic extracts of Zaung-yar at different concentrations (1.625, 3.125, 6.25, 12.5, 25, 50,100 and 200 μg/ml) were prepared for screening of radical scavenging activity. For comparison purpose, ascorbic acid (standard antioxidant) at concentrations (1.625, 3.125, 6.25, 12.5, 25, 50,100 and 200 μg/ml) were prepared and tested. Absorbance was determined at 517 nm by a UV spectrophotometer. The activity was usually expressed in terms of % inhibition. In addition, the average values of percent inhibition, IC_{50} (Inhibitory concentration 50%) were calculated by Linear Regressive Excel program.The % inhibition and IC_{50} values of ethanol extract is presented in Table 3. As shown in the table, the radical scavenging activity of ethanolic extract of *Averrhoa carambola* L. fruit has good IC_{50} value (IC_{50} =9.165 ppm) when compared to standard antioxidant, ascorbic acid (IC_{50} = 5.916 ppm). IC_{50} values of ethanol extract of *Averrhoa carambola* L. fruit is consistent with the reported value in literature (Felix. 2022).

Table 3. Absorbance of DPPH, % Inhibition and IC_{50} of Ethanolic Extract from the Fruit of *Averrhoa carambola* L. and Ascorbic Acid

Sample	Concentrations (µg/mL)	Absorbance	% Inhibition	IC_{50} (ppm)
Ethanolic extract of Zaung-yar fruit	1.625 3.125 6.25 12.5 25 50 100 200	0.257 0.228 0.209 0.197 0.144 0.125 0.106 0.059	34.27 42.26 48.20 52.06 66.75 73.45 80.51 85.09	9.165
Ascorbic acid	1.625 3.125 6.25 12.5 25 50 100 200	0.274 0.224 0.195 0.164 0.144 0.092 0.072 0.025	30.67 43.56 50.77 58.76 64.17 75.51 81.70 96.87	5.916

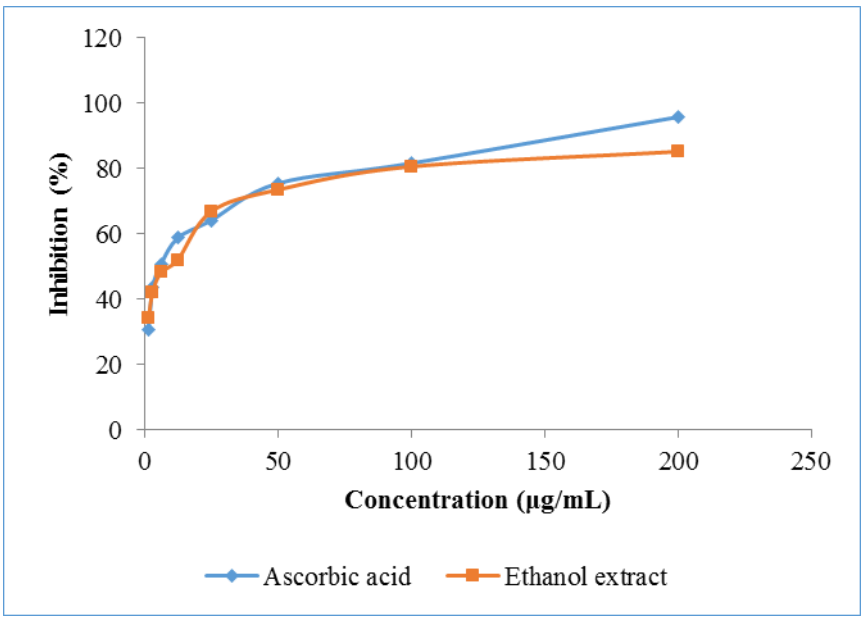


Figure 4 . Inhibition (%) of standard ascorbic acid and ethanolic extract of Zaung-yar fruit

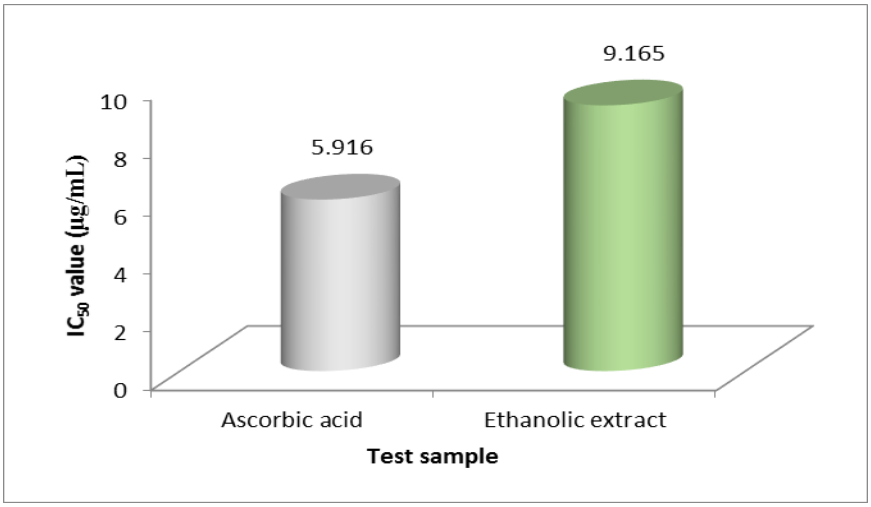


Figure 5. IC_{50}values of standard ascorbic acid and ethanolic extract of Zaung-yar fruit

Antimicrobial Activity of Zaung-yar (*Averrhoa carambola* L.) Fruit

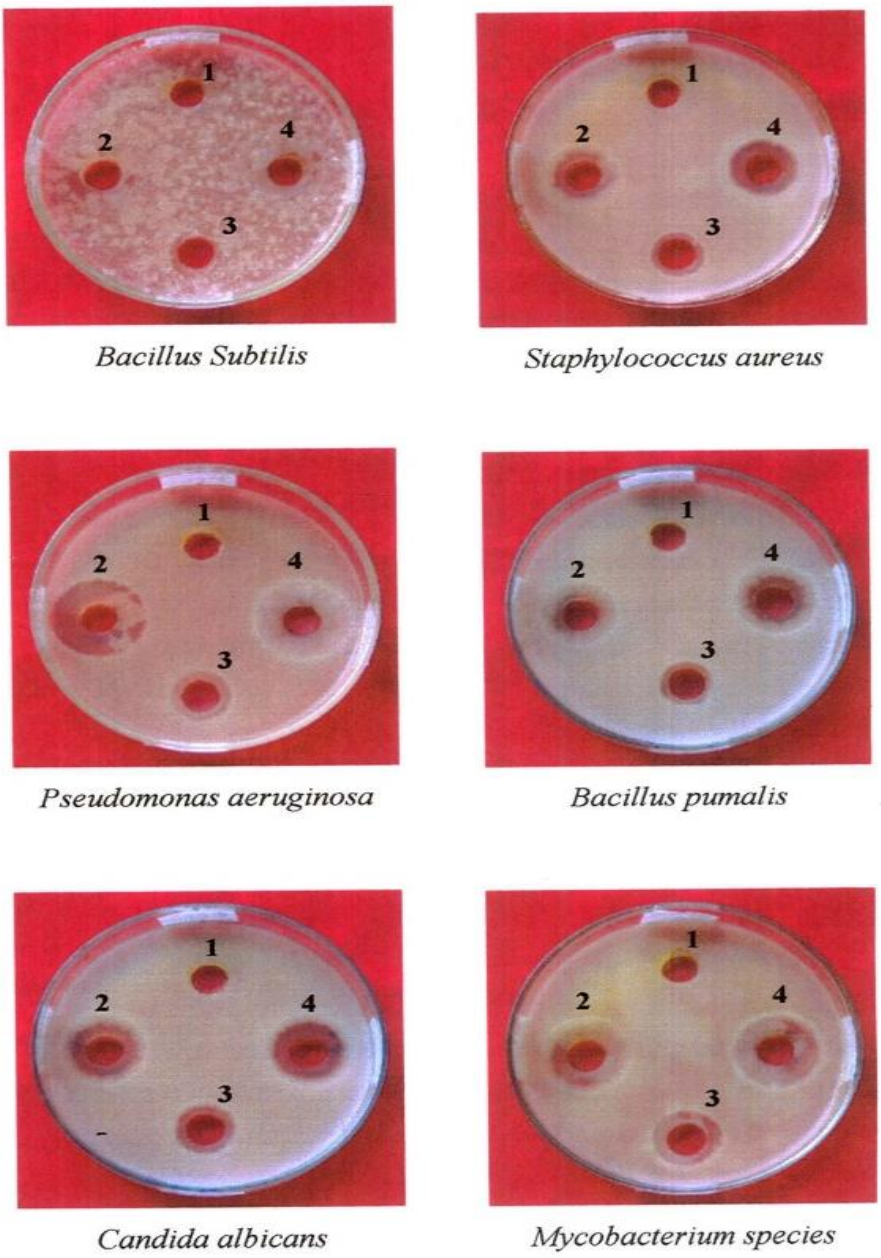
The 70% EtOH, PE, EtOAc and H_2O extracts of the fruit of (*Averrhoa carambola L.*) sample were tested for antimicrobial activity. It was observed that the (PE) extract of the fruit was inactive by agar well diffusion method on the six microbial species tested, namely *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis, Candida albicans* and *Mycobacterim* species, the extract of EtOAc, 70% EtOH and H_2O were moderately active. The photograph of inhibition zone diameters of different extracts against the six species of microorganism tested are shown in figure 8 and the results are summarized in Table 4. These expressed in millimeters, are the diameters of clear inhibition zones on agar plate formed around the cut agar well plate had previously been innoculated with test microorganism and the clear inhibition zones are due to the antibiotic activity of the extract placed in the agar well.

Table 4. Antimicrobial Activity of Different Crude Extracts of Zaung-yar Fruit (*Averrhoa carambala* L.) by Agar Well Diffusion Method

		Inhibition Zone Diameters (mm)					
No.	microorganism / Samples	1	2	3	4	5	6
1	Pet-ether extract	-	-	-	-	-	-
2	EtOAc extract	-	15 (++)	25 (+++)	12 (+)	15 (++)	20 (+++)
3	70% EtOH extract	12 (+)	15 (++)	15 (++)	14 (+)	15 (++)	15 (++)
4	H_2O extract	-	17 (++)	-	15 (++)	19 (++)	23 (+++)

Test Microorganisms
1. *Bacillus subtilis*
2. *Staphylococcus aureus*
3. *Pseudomonas aeruginosa*
4. *Bacillus pumilis*
5. *Candida albicans*
6. *Mycobacterium species*

Agar well – 10 mm
10 mm – 14 mm (+)
15 mm – 19 mm (++)
20 mm – above (+++)



1. PE extract
2. EtOAc extract
3. EtOH extract
4. H_2O extract

Figure 6. Inhibition zone diameters of different crude extracts of Zaung-yar fruit against six species of microorganism

Conclusion

From the overall assessment concerning with chemical and biological activity investigation on the fruit of *Averrhoa carambola* L., the following inferences may be deduced.

The Zaung-yar fruit possesses significant antioxidant activity when compared to standard antioxidant, ascorbic acid (IC_{50}= 5.916ppm). Activity of ethanolic extract was (IC_{50}= 9.165ppm). PE extract of the fruit was inactive by agar well diffusion method on the six microbial species.The extracts of EtOAc , 70% EtOH and H_2O

were moderately active. However, Zaung-yar fruit contains high oxalate content, so people who suffer kidney problems should not eat it. Zaung-yar fruit was observed to contain phenolic compound, glycosides, tannins, sponins, flavonoids, terpenoids and organic acid by phytochemical test. Six compounds 1 to 6 were isolated from the deacidified EtOAc extract by column chromatography on silica gel using PE : EtOAc (1 : 1.5) as eluent. The compound no.6 was characterized as abscisic alcohol by UV , FT IR and by reference to literature.

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