A Study on Preliminary Phytochemical Investigation and Nutritional Values of *Averrhoa carambola* L.

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Abstract

Averrhoa carambola L. of Oxalidaceae is native of South-East Asia and cultivated in some parts of India. The plant is distributed throughout Myanmar and the warmer regions of the world. The fruits are five-lobbed, fleshy and yellow-greenish. *Averrhoa carambola* L. is commonly known as star fruit. The fruits have great nutritional values, traditional medicine and numerous health benefits. The fruits are good source of antioxidants and used traditionally in mouth ulcers, toothache, nausea, diarrhea, ascites and asthma. The specimens were collected and identified with the help of available literature. The morphological characters of these plants were studied and described with the help of photographs. In preliminary phytochemical investigation, the presence of fruits showed that the presence of alkaloids, α-amino acid, carbohydrate, reducing sugars, glycosides, phenolic compounds, tannins and flavonoids, saponins and starch. The nutritional value that the presence of moisture was the highest percentage, carbohydrates were moderate percentages and proteins, crude fiber and fats were low. They were also important for nutritional perspectives. In this research, in green fruit extracts exceeding 4.782 mg/L and 4.724 mg/L in ripe fruit produced toxic less than 8000 mg/L (8 g/kg) in humans.

Keywords: morphological, phytochemical, nutritional values, elemental analysis

Introduction

Averrhoa carambola L. is a species of tree in the family Oxalidaceae; it has a number of common names, including carambola and starfruit. *Carambola* or star fruit, is the fruit of *Averrhoa carambola,* a species of tree native to the Philippines, Indonesia, Malaysia, Vietnam, Nepal, India, Bangladesh. *A. carambola* is commonly known as starfruit or carambola but other names include five fingers.*A. carambola* is a small tree or shrub that grows 5-12 metres tall, with rose to red-purple flowers. The flowers are small and bell- shaped, with five petals that have whitish edges. The tree is cultivated in tropical and semi-tropical regions for its edible fruits and its medicinal uses. The odour of the fruits resembles oxalic acid and their taste varies from very sour to mildly sweetish or sweetish. Preliminary phytochemical analysis of *carambola* fruit showed the presence of saponins, alkaloids, flavonoids and tannins (Guanghou & Leong, 2004).The entire fruit is edible. It may also be used in cooking and juice drinks. Edible fruit is a source of iron (low in calcium) and vitamins B and C, oxalate and potassium. Nutrient analysis of raw, fresh star fruit (per 100g) showed: energy 31 Kcal, carbohydrates 6.73g, protein 1.04g, total fat 0.33g. Fruits are generally eaten fresh, but occasionally used in desserts and juices as well. Becoming increasingly popular in Western markets, the star fruit is a pleasure. Star fruit comes in two varieties; sweet (with 5% sugars) and tart (with 1% acid). Unripe fruits can be ripened at room temperature until the fruits. One of the most important benefits of star fruit is reduced risk of cardiovascular diseases. This is because of the fruit's high content of polyphenol antioxidants. These antioxidants are also known to help in the prevention of cancer. The fruit helps in lowering cholesterol, constipation, jaundice. Its antimicrobial property, the fruit also infection caused by bacteria such as

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staphylococcus, salmonella, and *E.coli*. The present research was carried out with the aims and objectives: to verify the morphological identification of the star fruits, to analyze the phytochemical constituents, nutritional values and to give the knowledge of better health care for the people.

Materials and Methods

Collection and identification of *Averrhoa carambola* L.

The specimens of *Averrhoa carambola* L. were collected and identified from Yenanchaung Township in Magway Region from September, 2016 to January, 2017. Morphological characters of vegetative and reproductive parts of the specimens were identified by using the literature of (Hooker, 1885; Backer, 1965; Lawrence, 1969; and Dassanayake, 1987; Hindley and Chit KoKo, 1987 and Kress *et al*, 2003). The fruits of *Averrhoa carambola* L. were washed and cut into small pieces to dry faster. And then, both air-dried samples were pulverized and stored in air-tight containers to prevent moisture and contamination.

Preliminary Phytochemical Investigation of the fruits and seeds of *Averrhoa carambola*L.

For preliminary phytochemical investigation, the air-dried powders of the fruits were used. Tests for alkaloids, α-amino acids, carbohydrates, starch, reducing sugars, glycosides, phenolic compounds, saponins, tannins and flavonoids were done by using various solvents. These results were carried out according to the British Pharmacopoeia, 1968; Marrini Bettalo *et al*., 1981; Central Council for Research in Unani Medicine, 1987 and Trease and Evans, 2002.

Test for Alkaloids

The powdered sample (2 g) was boiled with 1%HCL (50 ml) for 20 minutes and filtered off. The filtrate was divided into two portions and tested with Dragendorff's reagent and Wagner's reagent. The precipitates treated with the above mentioned reagents showed the presence of alkaloid (Central Council for Research in Unani Medicine, 1987).

Test for α-amino acids

The powdered sample (2 g) was boiled with distilled water (25 ml) for 20 minutes and then filtered. And then a few drops of each filtrate were spotted on a filter paper by using a capillary tube. This paper with filtrate was allowed to dry and sprayed with ninhydrin reagent. It was dried at room temperature and then kept in oven at 110ºC for a few minutes. The colour of spots changes to the pink due to the presence of α-amino acids (Marrini Bettolo *et al*., 1981).

Test for Carbohydrates

The powdered sample (2 g) was boiled with distilled water (25 ml) for 20 minutes and then filtered. The filtrate was then placed into a test tube and a few drops of 10% α-napthol was added and shaken. The test tube was kept inclined at an angle of 45º and about 1 ml of concentrated sulphuric acid was slowly introduced along the inner side of the test tube. A red ring was formed between the two layers (Trease and Evans, 2002).

Test for Starch

The powdered sample (2 g) was boiled with distilled water (25 ml) for 20 minutes and then filtered. Two drops of iodine solution were added to this filtrate. Bluish black precipitates indicate the presence of starch (Central Council for Research in Unani Medicine, 1987).

Test for Reducing Sugars

The powdered sample (2 g) was boiled with distilled water (25 ml) for 20 minutes and then filtered. A mixture of Benedict's solution was added to this filtrate and boiled on water bath for a few minutes. Brick red precipitates indicate due to the presence of reducing sugars (Trease and Evans, 2002).

Test for Glycosides

The powdered sample (2 g) was boiled with distilled water (25 ml) for 20 minutes and then filtered. To this filtrate, 10% lead acetate solution was added. Yellow precipitates showed the presence of glycosides (Marrini Bettolo *et al*., 1981).

Test for Phenolic compounds

The powdered sample (2 g) was boiled with 1% hydrochloric acid (25 ml) for about 20 minutes and filtered. The filtrate was treated with 3% ferric chloride solution. The yellow precipitates appeared due to the presence of phenolic compounds (Marrini Bettolo *et al*., 1981).

Test for Saponins

The powdered sample (2 g) was put into a test tube and some distilled water was added into a test tube. The mixture was vigorously shaken for a few minutes. The frothing appeared due to the presence of saponins (Marrini Bettolo *et al*., 1981).

Test for Tannins

The powdered sample (2 g) was boiled with distilled water (25 ml) for 20 minutes and then filtered. To this filtrate, 3% ferric chloride solution was added. The observation indicated the greenish brown precipitates because of the presence of tannins (Central Council for Research in Unani Medicine, 1987).

Test for Flavonoids

The powdered sample (2 g) was boiled with 95% ethanol (25 ml) for 20 minutes and filtered. A few drops of concentrated hydrochloric acid and 0.5 g of Mg were added to this filtrate. Pink color appeared due to the presence of flavonoids (Central Council for Research in Unani Medicine, 1987).

Nutritional values of the fruits of *Averrhoa carambola* L.

The fruits of *Averrhoa carambola* L. were calculated for its nutritive values at Myanmar Food Processors and Exporters Association (MFPEA), Yangon. The nutritional values had been undertaken according to the Association of Official Analytical Chemists (AOAC) method (Horwitz, 1980).

Determination of protein content

The protein content was determined according to method in carbohydrate Chemistry, 1978. Each sample was accurately weighed from 0.1 g to 1 g into the digestion flask (kjedahl flask). One gram of copper sulphate and 10 g of anhydrous sodium sulphate and 10 ml of concentrated sulphuric acid were added one after

another. The flask was kept in an inclined position on a heating mantle and heated gently until the foaming ceases. A small amount of paraffin was added to reduce foaming. The solution was boiled vigorously until it was clear. The digestion flask was then cooled and about 200 ml of distilled water and 75 ml of sodium hydroxide solution were added into it. A few zinc granules were also added. The digestion flask was connected immediately to the distillation ball or trap on the condenser, and the tip of the condenser must be immersed in a 25 ml of standard acid (0.5 N or appropriate 0.1 N) in the conical flask. Then, the flask was rotated to mix the contents thoroughly and heated immediately, until all ammonia had distilled over (at least 150 ml distillate). The receiver must be lowered down first before distillation was complete and the tip of the condenser must be washed with distilled water. The excess acid was filtrated with standard 0.1 N sodium hydroxide using methyl red as an indicator by the method of A.O.A.C (Horwitz, 1980).

Protein content was then calculated by the following formula.

$$\% \text{ protein} = \frac{(x - y) \times 0.014 \times N \times 6.25 \times 100}{\text{wt. sample}}$$

Determination of fiber content

About 2 g of each sample was accurately weighed and introduced into a 500 ml flask. Then, 200 ml of 1.25% sulphuric acid was added. The contents were refluxed for 30 minutes and then filtered through linen clothe in a fluted funnel. The residue was thoroughly washed with hot distilled water until the washing was no larger acidic. The residue was washed back quantitatively into the flask with 100 ml of distilled water; 100 ml of 2.5% NaOH solution were added and refluxed exactly for 30 minutes. It was then filtered thoroughly with hot distilled water. Next it was washed with 1% HCL and again with hot distilled water till free from acid. The remaining water was squeezed out as far as possible. Finally, the residue was washed with a small amount of 95% ethanol. The residue was transferred quantitatively into a weighed crucible and dried to a constant weight in an oven at 105˚C for three hours. It was cooled in desiccators and weighed.

The crucible and contents were ignited in a muffle furnace (600˚C) until white ash was obtained. It was cooled and weighed. The difference in two weights represents the weight of crude fiber by the method of A.O.A.C (Horwitz, 1980).

$$\% \text{ Crude fiber} = \frac{\text{wt. of fiber (g)}}{\text{wt. of sample (g)}} \times 100$$

Determination of fat content

The fat content was determined by the soxhlet extraction method. Sample 50 g were placed in a thimble. The neck of thimble was plugged with a little cotton wool to prevent the sample from floating out. The thimble was introduced into a soxhlet extractor and the extractor was connected to the flask and condenser. For extraction, exactly 200 ml of petroleum ether was used. When the solvent colorless, the extraction was stopped, it took about 6 hours. The petroleum ether extract was concentrated to small volume and transferred to a weighed beaker. It was evaporated to dryness on water bath. The residue containing beaker was weighed. The different

weight of residue containing beaker was because of the weight of fat by the method of A.O.A.C (Horwitz, 1980).

$$\% \text{ non volatile residue (fat)} = \frac{\text{wt. of residue x 100 (g)}}{\text{wt. sample}}$$

Determination of carbohydrate

An accurately weighed 1g of samples was placed in a round bottom flask (500 cm^3), to which distilled water (200 cm^3) and hydrochloric acid (20 cm^3) were added. The mixture was then boiled gently under a reflux condenser for 2-5 hours, cooled and neutralized with sodium hydroxide (25 mol dm^3). The neutralized solution was then filtered into a volumetric flask (250 cm^3).The round bottomed flask was washed several times with distilled water and the washing added to the volumetric flask. The solution in the flask was made up to the mark with distilled water. An aliquots (3×10 cm^3) of the Fehling's solution was titrated with the above dilute hydrolyzed solution using methylene blue as an indicator. The amount of glucose calculated from the volumetric analysis multiplied by 0.93 as equal to the amount of starch by the method of A.O.A.C (Horwitz, 1980).

$$\text{Reducing sugar\% (Dextrose \%)} = \frac{\text{Second treatment volume x 20 ml of 0.5MHCl x sample wt.}}{\text{Make volume 100 or 200 x 0.3 x first treatment volume of value x 0.5 HCl}}$$

Carbohydrate % = Dextrose % × 0.93

Elemental analysis of *Averrhoa carambola* L. fruits by using AAS

Sample preparation

About 0.5 g various parts of the sample were accurately weighed (-80) and meshed into a dry clean 18x150 mm Pyrex test tube. Digested in 5 ml of HNO_3:HCl (1:4) concentrated acid mixture. Evaporated the solution to dryness overnight in an air. Leached the residue on a water bath tried with 10 ml of HNO_3 weak acid mixture at a temperature of about 70°C for 30 min. Stirred the solution, by using votex mixer. The resultant solution 10 ml was pipettes accurate and made up to 100 ml with deionized water again. The solution stood for overnight. The solution was aspirated on an atomic absorption spectrophotometer.

Determination of elements

In this work, the use of AAS instrument is Perkin Elma Analyst 800 spectrophotometer. Atomic absorption spectrophotometry consists of measuring the absorption radiation by the atomic vapour produced from the sample solution at a wavelength that is characteristic of the element being determined . The sample solution is aspirated into the flame that is irritated by light from a hollow cathode lamp that emits light of a wavelength characteristic of that element. The light is absorbed by the atoms of the element present in the flame. The degree of absorption is measured photometrically. Atomic absorption spectrophotometry is particularly suitable for analysis of principal mineral elements and trace elements.

Results

Botanical name	-	*Averrhoa carambola* L.
Myanmar name	-	Zaung-yar
English name	-	Star fruit; Caramobola Apple; Chinese Gooseberry; Coromandel Gooseberry;
Family	-	Oxalidaceae

Identification characters:

A small tree. Leaves alternate, unipinnately compound, exstipulate; petioles pulvinate; the margins entire, the tips acute. Inflorescences axillary paniculate cymes; bracts deciduous. Flowers ebracteolate, pedicellate, bisexual, actinomorphic, pentamerous, hypogynous. Calyx, 5 sepals, ovate glabrous, sepaloid. Corolla synpetalous, campanulate, the corolla lobes 5, obovate-oblong to oblog, the tubes cylindrical, purplish, pubescent. Androecium apostemonous, stamens 10, 5 fertile and 5 staminoses, basally connate, the staminodes shorter, the filaments dilated at the bases, the anthers dithecous, dorsifixed, introrse, dehiscence longitudinal. Pistil 1, ovary ovoid-oblongoid, 5-lobed, 5-carpelled, syncarpous, 5-loculed, the placentation axile, the ovule 1 in each locule, the styles 5, distinct, the stigmas terminal on each style, capitate. Fruit a berry, oblongoid 5-lobed, smooth; seeds ellipsoid, smooth, arillate.

Fig. 1. Habit

Fig. 2. Inflorescence

Fig.3. L.S of Flower

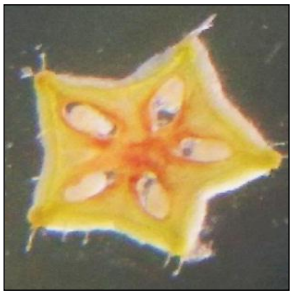

Fig. 4. T.S of Ovary

Fig. 5. Ripe Fruits

Fig.6. Green Fruits

Sensory Characters of the Powdered fruits *Averrhoa carambola* L.

The colour of powdered green fruit was pale brown and dark brown in ripe fruit. The odour was aromatic in green and ripe fruits. The taste of powdered green and ripe fruits were sour. The textures were granular in both samples. The results were shown in Figures (7, 8) and Table (1) .

Fig.7. Powdered Green Fruits

Fig.8. Powdered Ripe Fruits

Characters	Green	Ripe
Colour	Pale-brown	Dark-brown
Odour	Aromatic	Aromatic
Taste	Sour	Sour
Texture	Granular, Fibrous	Granular, Fibrous

Preliminary Phytochemical Investigation of the fruits of *Averrhoacarambola*L.

The results of these tests confirmed the presence of fruits of *Averrhoacarambola* L.showed the presence of alkaloids, α-amino acids, carbohydrates, reducing sugars, glycosides, saponins, phenolic compounds, tannins, flavonoids and starch. The results were shown in Figures (9, 10) and Table (2).

Table (2) Preliminary Phytochemical Investigation of the fruits of *Averrhoa carambola* L.

No.	Tests	Extracts	Test Reagents	Observations	Results
1	Alkaloids	1%HCL	Dragendroff's reagent	White ppt.	+
			Wagner's reagent	Reddish brown ppt.	+
			Mayer's reagent	White ppt.	+
2	α-amino acids	DW	Ninhydrin reagent	Pink spot	+
3	Carbohydrates	DW	10%α-napthol and Conc: H_2SO_4	Red ring	+
4	Starch	EtOH	Iodine solution	Bluish black	+
5	Reducing sugars	Dil H_2SO_4	Benedicts solution	Brick red ppt.	+
6	Glycosides	EtOH	10% lead acetate solution	Yellow ppt.	+
7	Phenolic compounds	EtOH	3% $FeCl_3$ solution	Yellow ppt.	+
8	Saponins	DW	Distilled water	Frothing	+
9	Tannins	EtOH	1% $FeCl_3$ solution	Greenish Brown ppt.	+
10	Flavonoids	EtOH	Mg/HCL	Pink	+

(+) present
(-) absent

Nutritional values of the fruits of *Averrhoa carambola* L.

The nutritional values revealed the presence of ash, carbohydrate, energy value, total sugar, moisture and acidity contents were higher in fruits except for protein, crude fiber, fat and ether extract. The results were shown in Table (3).

Table 3. Nutritional values of the fruits of *Averrhoa carambola* L.

No.	Test Parameter	Result %
		Fruits
1	Moisture	92.69
2	Ash	0.36
3	Protein	0.38g
4	Crude Fiber	0.809g
5	Fat	0.8
6	Carbohydrate	9.38g
7	Energy Value (Kcal/100g)	35.7g

Elemental Analysis by using AAS

In the present work, the content of As was (4.782 mg)in the green and 4.724 mg in the ripe fruits which were lower than the toxic level (8000 mg/L) but toxic Pb was non-detective in green and ripe fruits. Cd was trace elements in green and in ripe fruits. The experimental data were shown in Table (5).

Table 5. Elemental analysis (mg/L) in *Averrhoa carambola* fruits

Elements	Green	Ripe
Pb	0.457	0.434
As	4.782	4.724
Cd	0.119	0.068

Discussion and Conclusion

In this paper, morphological characters, sensory characters, phytochemical investigation, nutritional values of *Averrhoa carambola* fruits were carried out. In the morphological studies, the plant is small trees, leaves are alternate, ovate to ovate-oblong in shape. Purple colored flowers are produced in the axils of the leaves. The flowers are arranged in clusters and each cluster is attached to the tree with red stalks. The flowers are small, pedicellate with 5 petals and sepals. The fruits are green when small and unripe but turn to yellow or orange when matured and ripe. The fruits are fleshy with an oblong shape and the seeds are brown colored, flat and thin. These characters are in agreement with those described by (Hooker, 1885, Lawrence, 1964, Backer, 1965; Dassanayake 1987 and Pandey, 2000).

The fruits of *Averrhoa carambola*, which has been investigated from the point of view of medicinal aspects in this research. In preliminary phytochemical investigation, the presence of fruits showed that alkaloids, α-amino acid, carbohydrate, reducing sugars, glycoside, phenolic compound, tannin and flavonoids saponin and starch. These characters are in agreement with (British Pharmacopoeia, 1968 and WHO, 1998).

Averrhoa carambola L. (Zaung-yar) is popularly known by local people for its edible fruits and commercial values. It is cultivated as fruit plants and medicinal plants in some countries. In sensory characters, the powder of the fruits, the colour is pale-brown and ripe fruits, the one dark brown colour also observed. These characters are in accordance with (Metcalfe and Chalk, 1960, Pandey *et al*., 2011).

Thus, the star fruit is having high medicinal value and it may thus be considered an important gift from nature to mankind. Chang *et al*., (2002) stated that the nutritional value of fruit contains about consuming 100% of this fruit that can provide (Kcal 100g) 31g, protein 1.04g, carbohydrates 6.73g, dietary fibre 0.80g, fat 0.33g, iron 0.32-1.65 mg, phosphorus 15.5-21.0 mg, potassium 2.35 mg, oxalic acid 7.6 mg and citric acid 1.32 mg. In this research, the results of nutritional values, represent protein 0.38g, carbohydrate 9.38g, fat 0.8g, crude fiber 0.809g, moisture 92.69 g, ash 0.36 g and energy value (Kcal 100 g) 35.7g.

In this research, heavy mental contents in arsenic were 4.782 mg/L in green fruit and 4.724 mg/L in ripe fruit. The concentrations of lead and cadmium were low. The lead and cadmium concentrations in both the green and ripe fruits were above the (WHO, 1991) permissible level of 0.005 and 0.05 ppm, respectively. However, the high concentration of the lead and cadmium in both green and ripe fruits make the star

fruit because the concentrations were below than the recommended daily intake of these metals but consumers should be aware of taking green fruit as these amounts can be harmful if the fruits are taken in large quantities. Thus, *Averrhoa carambola* L. fruit was consumed suitable for human. It can be concluded that the plant has high medicinal value, no toxicity and it may thus be considered an important gift from nature to mankind.

For the future research, the fruits of *Averrhoa carambola* L. posses the medicinal value, therefore, the bioactive compounds should also be extracted. Moreover the bioactivity of the fruits of *Averrhoa carambola* L. for anti-inflammatory activity, hypoglycemic activity, hepatoprotective activity, antimicrobial activity, anti-ulcer activity and antioxidant activity should be investigated. These resources have the prospect of finding new clinically efficient antimicrobial or antioxidant compounds and the knowledge can be extended for future investigation into the field of pharmacology for better drug discovery.

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