

Assessment of Nutritional Value of *Limonia acidissima* L. (Wood Apple)

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Abstract

The objective of the study was to investigate *Limonia acidissima* L. (wood apple) to determine the moisture and ash content, total amount of carbohydrates, fatty acid compositions, the quantity of protein and micronutrients (Na, K, Zn, Fe) present in wood apple grown in Bangladesh. FT-IR spectroscopy was used to pinpoint many biologically significant functional groups. The moisture and ash content obtained were 74.02 and 25.44%, respectively. Carbohydrate content determined by ultraviolet-visible (UV-Visible) spectroscopic method was 16.14 g per 100 g of dried fruit sample. The fatty acids were identified by Gas Chromatography-Flame Ionization Detector (GC-FID). The relative percentage of bound fatty acids mainly palmitoleic acid, octadecanoic acid, cis-9-oleic acid and octanoic acid were 17.33, 15.09, 15.09 and 45.09%, respectively. The amount of protein was 5.11%. The micronutrients such as Na, K, Zn, Fe present in the edible portion of wood apple measured by atomic absorption spectroscopy (AAS) were 10.40, 58.24, 0.37 and 1.67 mg per 100 g of dried sample, respectively. The fruit has long been utilized in herbal medicines and is eaten raw or processed into various products to prevent noncommunicable diseases and micronutrient deficiencies.

Keywords : Atomic absorption spectroscopy, GC-FID, *Limonia acidissima*, micronutrients, UV-Visible spectroscopic method.

I. Introduction

Limonia acidissima L. (wood apple) belonging to the monotypic¹ genus *Limonia* is one of the underutilized fruits of Rutaceae family (Citrus family) and is mainly found in Bangladesh, India, Sri Lanka² and Southeast Asia because of suitable agro-climatic conditions. It is also known as kodbel in Bangladesh which is similar in appearance to the Bael fruit (*Aegle marmelos*). All parts of the plant have traditionally been used as a natural medicine to treat a variety of ailments. A tall, slow-growing deciduous³ tree with a few upward-reaching branches that curve outward near the summit and are divided into thin branchlets with drooping tips, the *L. acidissima* plant is native to the dry plains of Bangladesh. Its fruit has a spherical shape and a diameter of 5–12.5 cm⁴. The rind is 6 mm thick and has a hue that is greyish-white⁵. It has a woody, extremely hard outer shell (known as the rind), which is particularly challenging to crack open. Seeds 0.2-0.24 inch (5-6 mm) long, hairy, with thick, green cotyledons; germination epigeal⁶. In Malaya, flowering takes place in February and March, the leaves fall off in January, and the fruit ripens in October and November. The fruit ripens in Bangladesh from the beginning of October to March. The fruit is dropped onto a hard surface from a height of one foot to determine its maturity (30 cm). Fruits that are immature bounce, but adult fruits do not. Then, the fruit is left in the sun for two weeks after harvesting to allow it to fully mature⁷. The pulp has numerous little white seeds imbedded in it and is dark, mealy, fragrant, resinous, sour, or sweet-tasting. Drinking wood apple juice provides various stomach and digestive system benefits⁸. It aids in the treatment of digestive issues such as dysentery and

diarrhea. It is also recognized to be a good constipation cure that relieves discomfort. It has laxative properties as well.

The activity of adaptogens against blood impurities, leucorrhea, dyspepsia, and jaundice are among the biological activities of *L. acidissima*, which is considered a hepatoprotectant⁹. The fruit is also astringent, especially when unripe, and a cardiac tonic¹⁰. An alternate treatment for intestinal parasites like *Entamoeba histolytica* and *Ascaris lumbricoides* can be made from finely ground unripe fruit. There are a large number of phytochemicals i.e., secondary metabolites like alkaloids, anthocyanin, tannins¹¹ effectively used to cure a wide range of human diseases. Its sticky pulp can be used to make syrups, beverages and jellies. The fruit can be consumed raw, but because of its sour flavor, it must be sweetened. Wood apple contains a considerable amount of protein, carbohydrate, fatty acids, crude fiber, calcium, phosphorus, iron, zinc, copper, manganese, sodium, potassium, manganese and Vitamin C among others¹². This paper reports the investigation of moisture content, ash content, carbohydrate content, fatty acid compositions, protein content and micronutrients (Na, K, Zn, Fe) present in wood apples grown in Bangladesh.

II. Materials and Methods

Sample Collection

The wood apple, *L. acidissima*, fruit was collected from a local market in Dhaka, Bangladesh which a taxonomist identified. The fruit was then cleaned, peeled away from the hard outer shell and blended with seeds. Some portion

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of the blended material was kept for determination of moisture and ash content while the other portion was used for freeze drying. Then the dried wood apple was ground to make a mesh size of powder by a grinder and stored in an airtight bottle for further investigation.

Solvents and Reagents

Anhydrous CaCl_2 , $\text{BF}_3\text{-CH}_3\text{OH}$ complex (Merck, Germany), extra pure *n*-hexane, H_2SO_4 (98%, w/w), phenol (Merck, Mumbai, India), 60% NaOH (BDH, U.K), 10% $\text{Na}_2\text{S}_2\text{O}_3$ (w/v), 2% H_3BO_3 in double distilled H_2O , HCl (standard solution) and Tashiro's indicator (methylene blue (0.1%) and methyl red (0.03%) in ethanol) were used.

Apparatus

The moisture and ash content were determined by heating the sample in a carbolite oven (EYELA, NDO-450ND). Total carbohydrates and protein content were estimated by double beam UV spectrophotometer and a vortex machine was used to prepare standard glucose solutions. Fatty acid analysis was carried out by Gas Chromatography- Flame Ionization Detector (GC-FID). Micronutrient content was estimated by Zeeman atomic absorption spectrophotometer. For drying the fruit sample freeze dryer (LABCONCO) was used. Analytical balance, rotary evaporator and reflux condenser were also used throughout the process.

Extraction and Fractionation of Crude Extract

Powdered wood apple (10 g, pulp) was soaked in *n*-hexane (100 mL). The entire mixture was filtered through cotton after three days and the filtrate was then concentrated at a temperature below 40 °C using a rotary vacuum evaporator. The crude extract was dissolved in water (50 mL) and then *n*-hexane was added and the mixtures were transferred in a separating funnel. According to the affinity of the solvent systems, the components of the crude extract were split into two phases and the organic phase was separated for isolation of compounds.

Moisture and Ash Content

The analytical methods adopted by the AOAC (Association of Official Analytical Chemists) were used to determine moisture and ash content. For determination of moisture content and ash content wood apple sample was taken in porcelain crucible, and the initial weight of the crucible with and without sample was taken. The crucible was then kept in an oven at 105 °C for 2 hours. Again, the weight of the crucible containing the sample was measured which in calculation gives the percentage of moisture content. The crucible was further kept in the carbolite furnace at 700 °C for 4 hours, let stay overnight and transferred to desiccators to cool. The final weight of the crucible containing the sample was measured which in calculation gives the percentage of ash content.

Determination of Total Carbohydrate Content

Total carbohydrate content was determined following our published method¹³. The calibration curve of standard D (+)-glucose and the phenol-sulfuric acid test were used to evaluate the total amount of carbs in wood apple fruit samples.

Extraction of Fatty Material and Bound Fatty Acids Estimation

Sample (10 g) and 50 mL *n*-hexane was refluxed for about one hour and repeated for three times. The extracts were combined and filtered through anhydrous CaCl_2 . The filtrate was dried to a constant weight of oil (0.50 g) and 1 mL of alcoholic 0.5M NaOH was added. Then the flask was shaken and refluxed for 30 min at (95 °C). The aqueous layer was diluted with HCl and partitioned with *n*-hexane. The upper organic phase was collected and then evaporated until dry, and finally fatty acid analysis were carried out following the procedure described elsewhere¹⁴.

Determination of Micronutrients Content

Atomic absorption spectrometry based on a flame atomizer was used to evaluate micronutrients. The radiation source used was a hollow cathode lamp. Wood apple fruit sample (10 g) was dried at 100 - 105 °C. For ash content, the sample temperature was gradually increased from 100 - 600 °C in a muffle furnace then nitric acid (HNO_3) and water (1:1 v/v) mixture was added. Now the sample was heated (at a low flame) to remove carbonaceous matter left in the ash. After cooling, concentrated HNO_3 (a few drops) was used to completely dissolve the sample. Furthermore, the samples were transferred into a volumetric flask (100 ml) and up to the mark with H_2O (distilled). These stock solutions were used for minerals analysis by Atomic Absorption Spectroscopy (AAS)¹⁴.

III. Results and Discussion

The nutritional quality of a food product is determined by evaluating its proximate composition.

Moisture Content

A significant factor that determines the quality of fresh fruits and vegetables is their moisture content. The mean value of moisture content was $74.11 \pm 0.56\%$ in wood apple sample (Table- 1). In 2008, Chowdhury et al. reported that moisture content was 68.45% which is substantially similar to the current study¹⁵. In 2014, Gupta and Pandey *et al.* reported that the water content in wood apple sample was quite low (6.4%) which may be advantageous in view of increasing the sample's shelf life¹⁶. According to a 2013 analysis by Uyoh *et al.*, food's moisture content serves as an indicator for the microbial attack, making it sensitive and vulnerable to deterioration¹⁷.

Ash Content

Ash content is a measure of the food's nutritional value and an indirect indicator of important mineral content. The mean value of ash content determined in wood apples was $25.44 \pm 0.09\%$ (Table-1). In 2014, Pandey *et al.* reported that the ash content in dried pulp was 5.28% and in 2016, Hiremath *et al.* also reported the ash content in wood apple samples 1.55% that is a few amounts smaller than this study¹⁸. The ash content in the wood apple fruit was found to be high in this study, implying that wood apples contain more essential minerals for physical development.

Table 1. Determination of moisture and ash content of wood apple fruits (n = 3)

Parameter	Mean \pm SD (%)	RSD (%)
Moisture content	74.11 ± 0.57	0.77
Ash content	25.44 ± 0.09	0.35

Notes: SD-Standard deviation & RSD-Relative standard deviation

Carbohydrates

Carbohydrates provide energy in the body. Wood apples are rich in carbohydrates which contain both edible and inedible carbohydrates. Amount of total carbohydrate determined was 16.14 g / 100 g (Table-2). In 2016, Hiremath *et al.* reported that the total carbohydrate in wood apple was 20.66% which is comparable to the current research¹⁸. In 2014 Pandey *et al.* reported that 70% carbohydrate was present in wood apple. In 2022, Ulvie *et al.* reported that this fruit contained 5.87% carbohydrates and Hassan *et al.* (2022) reported that the fruits are rich in total carbohydrates ($24.74 \pm 0.19\%$)¹⁹.

Protein content

The human diet may benefit from wood apple's high content of some important amino acids even if they are not a strong source of protein. The protein content determined was found to be 5.11% (Table -2). In 2014, Pandey *et al.* reported that it was a good source of protein because it included a significant amount of protein (13.8%). In 2016, Hiremath *et al.* reported that wood apple contains 6.78% protein. In 2022, Yuliana *et al.* reported that wood apple contains 4.30% protein²⁰.

Table 2. Determination of carbohydrates, protein and fatty materials

Parameter	Amount (g per 100 g)
Carbohydrates	16.14
Protein	5.11
Fatty materials	0.50

Fatty Acids

The amount of fatty material of wood apple was found to be 0.50 g (Table- 2). The wood apple's fatty acids were transformed to their methyl esters and examined through GLC. By comparing the relative percentages of the fatty acids present in the sample with the retention times of the standard samples, the bound fatty acids present were identified. The relative percentage of bound fatty acids were found to be palmitoleic acid (17.33%), octadecanoic acid (15.09%), *cis*-9-oleic acid (15.09%) and octanoic acid (45.09%) which these are (Table-3) close enough to Ulvie *et al.* (2022) report. In 2014, Pandey *et al.* and Gupta *et al.* reported that fat content in wood apple was 4.38% which is quite low. In 2016, Hiremath *et al.* reported that fat content in wood apple was 1.38%. The crude fat content in wood apple fruit pulp was $0.99 \pm 0.01\%$ and the fatty acid profile represents the higher amount of unsaturated fatty acids. The fruitsthat are rich in contents of unsaturated fatty acids are valuable sources for treating various ailments. The crude extract was thoroughly mixed with powdered potassium bromide (KBr) and made into pellets under high pressure. The prepared KBr pellet was inserted into the FT-IR spectrophotometer (Shimadzu FT-IR 4800S) and experiment was recorded. The FT-IR spectrum of crude sample includes O-H (hydrogen bonded), C=O, C-O and C=C stretching peaks which indicate the presence of alcoholic, acidic or carbonyl type compounds. Spectrum of the sample contains C=C stretching vibration which may be conjugated with C=O.

Table 3. Retention time and relative percentages of fatty acids

Name of fatty acids	Retention time	Relative percentage (%)
Octanoic acid	05.61	45.09
Palmitoleic acid	32.21	17.33
Octadecanoic acid	39.20	15.09
<i>Cis</i> -9-oleic acid	40.57	15.09

Micronutrients

Micronutrients are chemicals that are only necessary in trace amounts but help the body grow, develop and maintain itself. Vitamins and minerals are essential for the release of energy from carbohydrates, proteins, and lipids (calories). Among 16 essential minerals Na, K, Fe and Zn were determined in wood apple by atomic absorption spectrometer. Na, K, Zn and Fe were found to be 10.40, 58.24, 0.37 and 1.67 mg per 100 g of edible portion of fruits, respectively. Potassium levels in wood apples were comparatively high that is beneficial for maintaining the neurological system in balance and sustaining its function. The low Na: K ratio made this fruit a food that people with hypertension might enjoy²¹. Though zinc content is low in

wood apple but it has anticancer properties. Wood apples are regarded as a beneficial dietary supplement for treating anemia and iron deficiency.

Micronutrients are chemicals that are only necessary in trace amounts but help the body grow, develop and maintain itself. Wood apple contains 1.67 mg zinc per 100 g of edible portion. Zinc has many diverse activities in the body as an essential trace element. It is necessary for a healthy metabolism and immune system, as well as for maintaining the appearance of skin and hair, aiding wound healing, improving sense of taste, smell and fertility. Zinc is contained in every cell in the body. It improves the immune system's defense against germs and viruses. Zn is required by the body for the production of proteins and DNA, which is the genetic material found in all cells. Zn is also required for optimal growth and development during pregnancy, infancy and childhood. In a nutshell, zinc is essential for good health²².

The human body needs iron for growth and development. Consuming wood apple can be a good source of iron (0.37 mg per 100 g). Hemoglobin, a protein found in red blood cells that transports oxygen from the lungs to all areas of the body, and myoglobin, a protein that distributes oxygen to muscles, are both made using iron. Iron is important for a variety of bodily activities, including general energy and attention, gastrointestinal processes, the immune system, and body temperature regulation. Potassium helps regulate fluid balance, muscle contractions and nerve signals. As wood apple is rich in potassium (58.24 mg per 100 g), a high-potassium fruit may help reduce blood pressure and water retention, salt sensitivity, protect against stroke and prevent osteoporosis and kidney stones.

Wood apple is one of a good source of essential nutrient sodium (10.40 mg per 100 g). The body requires sodium in small amounts (assuming no excessive perspiration) to maintain a balance of body fluids and keep muscles and nerves functioning properly. Sodium has many health benefits, including managing fluid levels, reducing sunstroke, increasing brain function and alleviating muscle cramps.

IV. Conclusion

Fresh wood apple fruits were found to contain moisture content 74.11% and ash content 25.44%. Amount of total carbohydrates and protein were found be 16.14 and 5.11%, respectively. The relative percentage of four fatty acids in wood apple fruits was found to palmitoleic acid (17.33%), octadecanoic acid (15.09%), *cis*-9-oleic acid (15.09%) and octanoic acid (45.09%). The micronutrients in this fruit are crucial for metabolism, the production of red blood cells, cell growth, and other physiological processes. The amounts of micronutrients (sodium, potassium, iron and zinc) were found to be 10.40, 58.24, 0.37 and 1.67 mg per 100 g of fruit sample. Therefore, wood apples not only

delicious with sweetish or sour taste but also as a phenomenal food that offers a variety of essential nutrients and other associated health advantages.

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