Nutritional Compositions, Antioxidant and Antimicrobial Activities of Exotic Fruit *Limonia acidissima* L.

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Abstract

Limonia acidissima L., Wood apple, is an edible fruit of family Rutaceae. It belongs to monotypic genus *Limonia*, confined to India, Pakistan, Sri Lanka and Southeast Asia. In central parts of Myanmar, this is locally called Thi. This plant is given as a medicine for the treatment of various disorders. The present study deals with nutritional compositions, antioxidant and antimicrobial activities of fruit pulp and fruit juice were investigated. The nutritional compositions of fruits pulp determined by AOAC method indicated that the percentage concentration of moisture, ash, crude protein, crude fibre, crude fat, carbohydrate were 78.62%, 1.55%, 3.76%, 2.95%, 2.50% and 10.62% respectively. The antioxidant activity of ethanolic extract was screened by DPPH with ascorbic acid as a standard indicated that IC_{50} value were 12.238 µg/mL and 8.23µg/mL. Agar-well diffusion method was used for antimicrobial activity. Ethanolic extract of fruit pulp was found to possess highest antimicrobial activity against *Candida albicans* followed by *Agrobacterium tumefaciens*. This study demonstrates that the fruits may be used as health promoting benefits.

Key words: *Limonia acidissima*, fruit, nutritional, antioxidant, antimicrobial

Introduction

Limonia acidissima L. is a tropical fruit plant of Rutaceae family and well-known for its medicinal properties. It is a religious tree planted in temples and gardens. This plant is given as a medicine for the treatment of various disorders. The valuable parts of the plant include its roots, fruits, bark and the leaves which are used for various therapeutic purposes. Syrups, drinks, jellies and jams can be prepared from its sticky pulp (Pandey *et al*., 2014).

It is a deciduous, slow-growing, erect tree with a few upward-reaching branches bending outwards near the summit where they are subdivided into slender branchlets drooping at the tips. The leaves are deciduous, alternate, dark-green and leathery. The spines are axillary, short and straight on some of the zigzag twigs. Flowers are small, numerous, dull- red or greenish, born in small, loose, terminal or lateral panicles. The fruit is berry with a hard, woody rind, which is grayish-white, scurfy rind about 6 mm thick. The pulp is sticky brown, aromatic odorous, resinous, astringent, acid or sweetish, white seeds scattered through it (Vijayvargia and Vijayvergia, 2014).

Wood apple fruit contains flavonoids, glycosides, saponins and tannins. There are reports that some coumarins and tyramine derivatives were also isolated from the fruits of *Limonia* (Ilango and Chitra, 2009). It is a nutrient rich fruit which contains a surprisingly high amount of protein and low levels of sugar and carbohydrates compared with many other fruits. *L. acidissima* L. is rich in Beta carotene, a precursor of vitamin A which also contains significant quantities of the B vitamins such as thiamine and riboflavin and vitamin C (Vijayakumar *et al*., 2013). Many phytochemicals are antioxidants including carotenoids and flavonoids. Antioxidants are very important to human health because they play a vital role in the risk of related oxidative damage associated with many clinical conditions and playing important role in many chronic diseases **(**Abdollahi *et al*., 2004). In recent years, there has been a

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growing interest to evaluate plants posing antimicrobial activity (Cowen, 1999). The fruits and stem bark of *L. acidissima* L. possess larvicidal and antimicrobial activity (Pandey, 2014). Ahamed *et al.*, **(**2008) screened the fruit pulp of *L. acidissima* L. for anti-inflammatory, antipyretic and analgesic activities as well as for the anthelmintic activity of its leaves.

Traditionally, all parts of the plants are given as natural medicine as a cure for various ailments. It is very often used against snakebites. People use it as a tonic for liver and heart, in diarrhoea and dysentery. The fruit is considered to be an effective treatment for hiccups and for problems of throat and gum. The pulp is applied onto bites and stings of deadly insects. It is also protective against skin cancer as it can protect UV rays. A paste "Thanaka", prepared from the pulp of *L. acidissima* L., is used as face cream to remove small spots and lesions on the skin (Pandey, 2014).

The aim of the present study is to know the valuable information of *L. acidissima* L..To achieve this aim, the objectives are to determine the nutritional compositions, antioxidant and antimicrobial activities of exotic fruit of *L. acidissima* L..

Materials and Methods

Sample collection

Freshly fruits of *L. acidissima* L. were collected from University of Mandalay, during the fruiting period of 2016 to 2019. They were identified with the comparison of literatures (Hundley and Chit Ko Ko, 1987 and Kress and Yin Yin Kyi, 2003).

Preparation of fresh pulp and fruit juice

The fruits were washed thoroughly with water to remove dust, the pulps were shed-dried and the juices were mechanically squeezed from the fresh fruit. The juices were filtered and the clear fruit juice was collected in clean containers.

Determination of nutritional compositions of fruit pulp

The experimental work for the nutritional compositions of fruit pulp of *L. acidissima* L. was carried out at the Food Industries Development Supporting Laboratory (FIDSL), Myanmar Food Processors and Exporters Association (MFPEA), Yangon, Myanmar. The nutritional value has been undertaken according to the Association of Official Analytical Chemist (AOAC) method (Horwitz, 1980).

Determination of antioxidant activity of fruit juice by DPPH Radical Scavenging Assay

The antioxidant activity of fruit juice was evaluated by using DPPH (1,1-diphenyl-2-picrylhydrazyl) Radical Scavenging Assay (Blosis, 1958 and Brand-Williams *et al*., 1995). This assay has been widely used to evaluate the free radical scavenging activity. The tests were conducted at Department of Chemistry, University of Mandalay.

DPPH free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol. This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colourless ethanol solution. The use of DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry.

This method based on the capacity of test sample to inhibit stable free radicle that was followed by decrease in absorbance at 517 nm. On the basis of absorbance value, % inhibition of sample extract and standard ascorbic acid were calculated and from which IC_{50} values were derived and reported.

Preparation of Reagents

In this experiment, three solutions were prepared. They are DPPH solution, standard solution and various concentrations of sample solution.

Preparation of 60 µM DPPH Solution

DPPH (0.0023 g) was thoroughly dissolved in 95% ethanol (100 mL). This solution was freshly prepared in the brown coloured flask and kept in refrigerator for no longer than 24 hrs.

Preparation of Standard Solution

Ascorbic acid (10 mg) was dissolved in 100 mL of 95% ethanol and it was used as a standard solution.

Preparation of Test Sample Solution

100 µL of sample was dissolved in 10 mL ethanol (Analar grade). This solution was thoroughly mixed at room temperature for 15 min to obtain 10 µL/mL sample solution. The concentrations of standard solution (10, 5, 2.5, 1.25 and 0.625 µL/mL) was determined by using two-fold dilution method. All solutions were thoroughly mixed for about 15 min at room temperature. The reduction of the DPPH radical was determined by reading the absorbance at 517 nm. The percent of inhibition of oxidation was calculated using the following formula:

% inhibition of oxidation = [(A – B) / A] x 100 %

A = Absorbance of DPPH solution

B = (Absorbance of sample + DPPH solution) – Absorbance of blank

Finally, IC_{50} value was determined by using linear regressive excel program. The half maximal inhibitory concentration IC_{50} is a measure of the effectiveness of a substance in inhibiting of a specific biological or biochemical function.

Determination of Antimicrobial activities of fruit pulp

Test microorganisms

Seven microorganisms were used in this study; three were gram positive bacteria; *Staphylococcus aureus* and *Bacillus pumilus and Bacillus subtilis* and three were gram negative bacteria; *Escherichia coli, Pseudomonas fluorescens* and *Agrobacterium tumefaciens* and a fungal strain was *Candida albicans* by using agar-well diffusion method at the Department of Chemistry, Meiktila University.

Results and Discussion

Scientific name - *Limonia acidissima* L. (**Figure: 1**)
Myanmar name - Thi
English name - Wood apple
Family - Rutaceae

Figure (1): *Limonia acidissima* L. fruits and fruit pulp

Determination of nutritional compositions of fruit pulp

The nutrient contents of fruits pulp of *L. acidissima* L. determined by AOAC method indicated that the percentage concentrations of moisture, ash, protein, fibre, fat and carbohydrate were 78.62%, 1.55%, 3.76%, 2.95%, 2.50% and 10.62% respectively (**Table 1**). According to present data, it can be observed that the fruit sample contain many nutritional values which have benefits to humans. Among them, moisture content was found to be the highest value (78.62%). Fruit pulp was found to be very rich in carbohydrates (10.62%). Sharma *et al*., (2014) shown that fresh pulp contained moisture 75.16%, ash 1.31%, protein 2.0% and fat 3.55%. Vijayvargia *et al*., (2014) stated that carbohydrates are probably the most abundant and widespread organic substances in nature, and they are essential constituents of all living things. Carbohydrates serve organisms as energy sources and as essential structural components. Proteins are the beginners and builders of biochemical reactions. Essential body processes such as water balancing, nutrient transport, and muscle contractions require protein to function.

According to present results, there was an appreciable amount of protein (3.76% by wt) making it a good source of protein, while its fiber content is also good. There is evidence that dietary fiber has a number of beneficial effects related to its indigestibility in the small intestine. Pulp has low amount of fat (2.50%) which makes it an ideal diet for overweight people. The energy value of was calculated and the value obtained was 80.0 Kcal. According to analysis in India, food value per 100g of edible pulp (ripe): moisture, 74.0%; protein, 8.00%; fat, 1.45%; carbohydrates, 7.45%; ash, 5.0%; calcium, 0.17%; phosphorus, 0.08%; iron, 0.07%; and tannins, 1.03% (Pandey, 2014).

Table (1): The result of nutritional compositions of fruit pulp

No.	Test parameter	Results
1.	Moisture	78.62%
2.	Ash	1.55%
3.	Crude Protein	3.76%
4.	Crude Fibre	2.95%
5.	Crude Fat	2.50%
6.	Carbohydrate	10.62%
7.	Energy value (Kcal/100g)	80.00

Determination of antioxidant activity of fruit juice by DPPH Radical Scavenging Assay

Antioxidant activity of freshly fruits juice of *Limonia acidissima* L. was estimated by measuring the DPPH radical scavenging activity of different concentration. Determination of absorbance was carried out at wave length 517 nm using spectrophotometer.

According to the results of percentage of radical scavenging activity (% RSA), from freshly fruits juice of *L. acidissima* L. are 47.79, 41.73, 40.59, 38.63 and 37.32 at various concentration of 10, 5, 2.5, 1.25 and 0.625 μg/mL respectively. IC_{50} value is 12.238 μg/mL (**Table 2 & Figure 2**). IC_{50} value of ascorbic acid is 8.23 μg/mL (**Table 3 & Figure 3**).

Shermin *et al*., 2012 stated that, the chloroform soluble fraction (CL) of crude methanolic extract showed the highest free radical scavenging activity with IC_{50} value 18.8 µg/mL. At the same time the pet ether soluble fraction (PE) also exhibited strong antioxidant potential having IC_{50} value of 37.64 µg/mL. According to Vijayvargia, 2014, the crude methanol extract of the stem bark of *L. acidissima* L. and its different organic soluble partitionates were screened for antioxidant activities. The methanolic extract of *Limonia* fruit was also screened for their free radical scavenging properties by Ferric reducing antioxidant power (FRAP) assay and DPPH radical scavenging assay.

Table (2): % Inhibition of various concentrations and IC_{50} value of fruit

Sample Concentration (µg/mL) (10^3)	Mean Absorbance	Mean % Inhibition	IC_{50} (µg/mL)
10	0.319	47.79	12.238
5	0.356	41.73	
2.5	0.363	40.59	
1.25	0.375	38.63	
0.625	0.383	37.32	

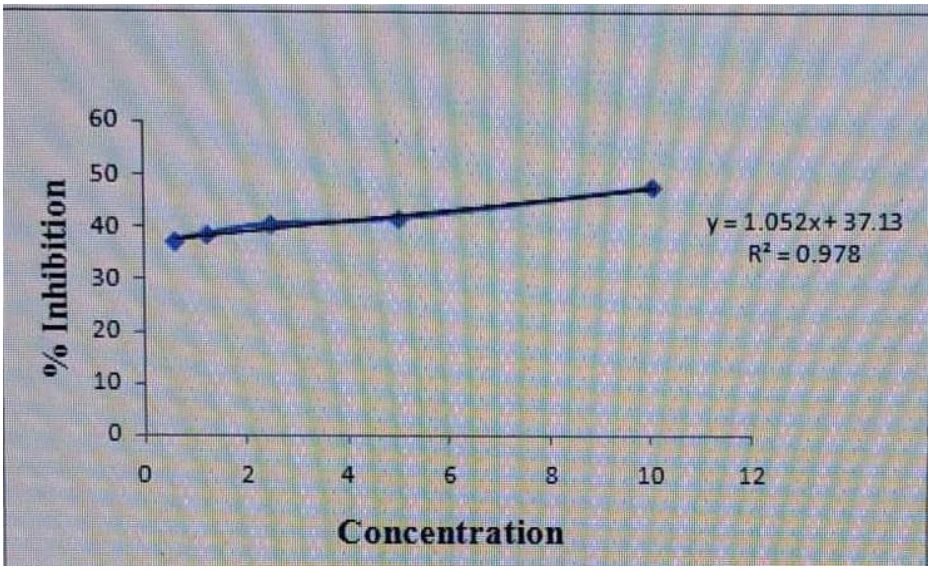

Figure (2): Plot of concentration Vs % inhibition of ethanol extract of fruit

Table (3): Radical scavenging activity of various concentrations and IC_{50} value of standard ascorbic acid

Sample Concentration (µg/mL)	Mean Absorbance	Mean % Inhibition	IC_{50} (µg/mL)
50.0	0.168	82.80	8.23
25.0	0.279	71.44	
12.5	0.401	59.06	
6.25	0.506	48.21	
3.125	0.608	37.77	

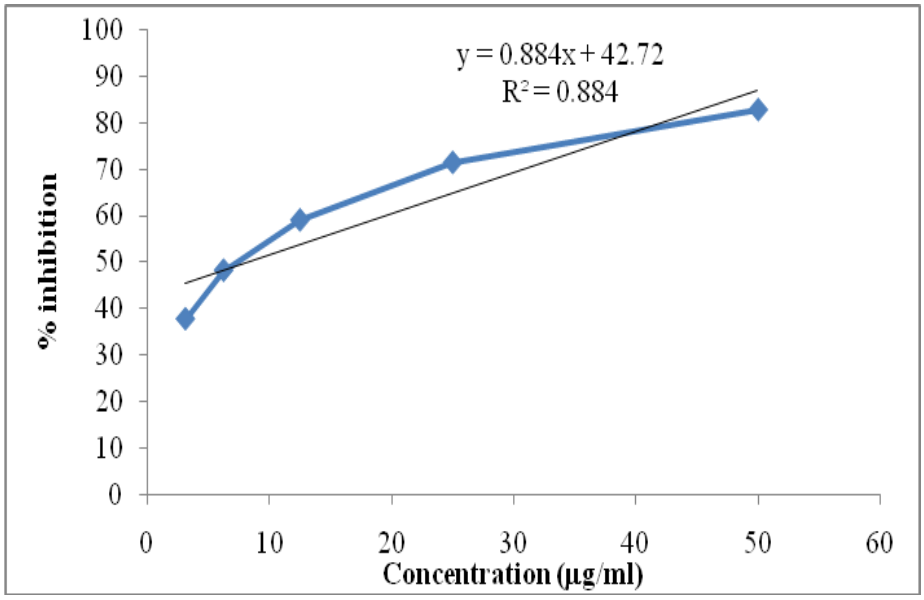

Figure (3): Plot of concentration Vs % inhibition of standard ascorbic acid

Determination of antimicrobial activities of fruit pulp

According to results, Wood apple fruit pulp of ethanolic and water extracts showed the significant antimicrobial activity against *Candida albicans* (52.00 mm and 32.42 mm), *Agrobacterium tumefaciens* (31.37 mm and 30.32 mm), *Bacillus subtilis* (29.52 mm and 19.88 mm), *Pseudomonas fluorescens* (29.49 mm and 22.81 mm), *Escherichia coli* (29.40 mm and 24.95 mm) and *Staphylococcus aureus* (28.35 mm and 31.94 mm). The ethanolic extract was found the lowest antibacterial activity against of *Bacillus pumilus* (11.39 mm) and water extract was not shown any inhibition zone. The results were compared with control and shown in **Table 4**.

Pandy, (2014) showed that antibacterial activity of dried pulp was assessed using agar well diffusion method against microorganisms. The methanolic extract of

pulp was found to possess highest antibacterial activity against *Staphylococcus epidermidis* followed by *Staphylococcus aureus* and *Bacillus subtilis.* Thomas, (2005) showed that the methanolic extracts of *L. acidissima* L. plant parts were tested against *Escherichia coli* and *Staphylococcus aureus* using disc diffusion method. The extracts from different parts showed varying degrees of antimicrobial activity.

Table (4): The results of antimicrobial activities of fruit pulp

No.	Microorganisms	Inhibition Zone Diameter (mm)		Control	
		EtOH	Water	EtOH	Water
1.	*Staphylococcus aureus*	28.35 (+++)	31.94 (+++)	-	-
2.	*Bacillus pumilus*	11.39 (+)	-	-	-
3.	*Bacillus subtilis*	29.52 (+++)	19.88 (+++)	-	-
4.	*Escherichia coli*	29.40 (+++)	24.95 (+++)	-	-
5.	*Pseudomonas fluorescens*	29.49 (+++)	22.81 (+++)	-	-
6.	*Agrobacterium tumefaciens*	31.37 (+++)	30.32 (+++)	-	-
7.	*Candida albicans*	52.00 (+++)	32.42 (+++)	-	-

(+) present (-) absent

Agar well- 8mm 8mm – 12 mm (+) 13mm – 17 mm (++) 18mm above (+++)

Conclusion

In conclusion, *L. acidissima* L. was analyzed for nutritional compositions, antioxidant and antimicrobial activity for use as functional foods and nutraceutical and flavoring agents to provide health benefits. In addition, it can be used as a food ingredient to make processed products like jams, jellies, sweets and savory chutneys. The results also support beneficial health claims and the fruit was rich in nutritional values and possessed antioxidant and antimicrobial activity. Thus, there is enormous scope for future research and further pharmacological investigation on *L. acidissima* L..

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References

Abdollahi M., Ranjbar A., Shadnia, S., Nikfar, S. & Rezaiee, A. 2004. Pesticides & Oxidative stress: A Review. Med. Science Monit. Vol.10: 141-147.

Ahamed, S.M., Swamy S.K., Jayaverra K.N., Rao J.V.,& Kumar V.S. 2008. Anti-inflammatory antipyretic and analgesic activity of methanolic extract of *Feronia limonia* fruit pulp. Pharmacologyonline; 3:852-857.

Blosis, M. S. 1958. Antioxidant Determinations by uses of Stable Free Radical. Nature, Vol. 26; 1199-1200.

Brand-Williams, W., Cuvelier, M.,& Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft und- Technologie. 28: 25-30.

Cowan, M.M. 1999. Plant product as antimicrobial agent of clinical Microbiology Review, Vol.61: 564-582.

Horwitz, W. 1980. Official Method of Analysis of the Association of Official Analytical Chemists. 3rd. Edition. Washington DC. Benjamin Franklin Station.

Hundley, H.G., & Chit Ko Ko. 1987. List of Trees, Shrubs, Herbs and Principle Climbers. Third Revised and Enlarged, SUPDT, GOVT Printing and STATY., Union of Burma, Rangoon.

Ilango, K., & Chitra, V. 2009.Antidiabetic and antioxidant activity of *Limonia acidissimia* Linn. in alloxan induced rats. Der Pharmacia Lettre.1: 117-125.

Kress, W.J., Defilipps R.A., Farr E & Daw Yin Yin Kyi. 2003. A Checklist of the Trees, Shrubs, Herbs, and Climbers of Myanmar. Department of Systematic Biology-Botany, National Museum of Natural History, Washington D. C.

Pandey, S., Gouri S., & Rajinder K.G. 2014. Evaluation of nutritional, phytochemical, antioxidant and antibacterial activity of exotic fruit "*Limonia acidissima"*. Journal of Pharmacognosy and Phytochemistry 2014; 3 (2):81-88.

Sharma, H. P., Patel, H., Sharma, S., & Vaishali. 2014. Study of physico-chemical changes during wood apple, *Feronia limonia* maturation. Journal of food research and technology.Vol.2, Issue 4: Pages: 148-152.

Shermin, S.,Fahima A.,Monira A., & Choudhury M.H. 2012. Antioxidant and Cytotoxic Activity of *Limonia acidissima* L..Dhaka Univ.J.Pharm. Sci. 11(1):75-77.

Thomas, A., & Ponnammal, N.R.. 2005. Priliminary studies on phytochemical and antibacterial activity of *Limonia acidissima* L. plant parts. Ancient Sci Life. Vol.25: Pages:57-61.

Vijayakumar, T.P., Punltha, K. & Banuprlya, L. 2013. Drying characteristics and quality evalution of wood apple , *Limonia acidissima* L. fruit pulp powder. Int. J. Cur. Tr. Res.2 (1):147-150.

Vijayvargia, P., & Rekha V. 2014. A Review on *Limonia acidissima* L.: Multipotential Medicinal Plant. Int. J. Pharm. Sci. Rev. Res., 28; Article No. 36, Pages: 191-195.