Comparison of Antimicrobial and AntioxidantActivities of Fresh Juices of *Citrus maxima* (Burm.)Merr. (Pomelo)and *Citrus paradisi* Macfad. (Grapefruit)

Seine Nyoe Nyoe Ko[1]

drseinennko@gmail.com

Abstract

Citrus fruits, belong to family Rutaceae, are rich source of active compounds and beneficial for human health. In the present study the antimicrobial and antioxidant activities of the juice of *Citrus maxima* (Burm.) Merr. And *Citrus paradise* Macfad have been investigated. Antimicrobial activity of fruit juices were followed by Agar Well Diffusion Method and comparing the clear inhibition zone. In *Citrus maxima*, the inhibition zone diameters were 11 mm in *Escherichia coli* and 15 mm in *Bacillus cereus*. In *Citrus paradisi*, the inhibition zone diameters were 15 mm in *Escherichia coli*, *Salmonella typhi* and *Bacillus cereus*. Medium activity was seen in case of *Bacillus cereus* where the inhibition zone diameter was 15 mm on both fruit juices. Antioxidant activity of fruit juices were carried out by using DPPH radical scavenging assay. IC_{50} value was 7.97 µl/ml in *Citrus maxima* and 3.01 µl/ml in *Citrus paradisi.* The results indicated that the Citrus fruit juices contain significant antimicrobial and antioxidant activity.

Key words: Citrus; Antimicrobial; Antioxidant; DPPH

Introduction

The genus *Citrus*L. of the family Rutaceae, is one of the most essential commercial fruit cropswhich are grown in all continents of the world (Tao *et al*., 2008).It is widely grown in the tropical and subtropical areas of the world. Citrus fruits are well-accepted by consumers of all over the world because of their attractive colours, pleasant flavours and aroma. Citrus fruits have now become an important dietary source of nutrients for people (Mehl*et al*., 2014).

Citrus fruits are in great demand as dessert fruits and also as preserves in the form of squash, cordial, marmalade or pickle. They can be eaten fresh like the sweet orange, mandarins, pomelo and the grapefruit or their segments may be canned. Citrus juices are consumed majorly because of their nutritional value and special flavour. They are rich in vitamin C, minerals and possess distinct flavour. Citrus fruits and

[1] Dr., Professor, Department of Botany, University of Myitkyina

juices have long beenrecognized to contain secondary metabolites including antioxidants such as ascorbic acid, flavanones, phenolics and pectin that are important to human nutrition (Munwar*et al*., 2015).

An antimicrobial is a substance that kills or inhibits the growth of microorganism such as bacteria, fungi or protozoans. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). Disinfectants are antimicrobial substances used on nonliving objects (Abirami*et al*., 2013).

Oxidative stress is believed to be involved in causation of more than hundred chronic diseases such as cancer, diabetes, cardiovascular and degenerative diseases etc.. One of the possible ways to fight these diseases is to reinforce our body's antioxidant defense system. Antioxidants possess the ability to scavenge the free radicals and protect the body from damages caused by the oxidative stress.

Fruits are important part of a healthy diet and are rich in vitamins, fibres, minerals, micro nutrients and phenolic compounds. Fruits have very high antioxidant value in comparison to vegetables and cereals. Fruits are also routinely consumed as fresh juices due to their suitability for ingestion which supply a relevant part of intake of health promoting polyphenolic phytochemicals. Citrus fruits are known for their high ascorbic acid content, a natural antioxidant that protects the organism from oxidative stress.(Al-Musharfi*et al*., 2015).

Citrus fruits are rich sources of useful phytochemicals, such as vitamins A, C and E, mineral elements, flavonoids, coumarins, limonoids, carotenoids, pectins, and other compounds. These phytochemicals, consumed through fresh fruits or their derived products, have been suggested to have a wide variety of biological functions including antioxidant, anti-inflammation, anti-mutagenicity, anti-carcinogenicity and anti-aging to human health (Zou*et al*., 2016).

Citrus maxima (known as Pomelo) exhibits some similarities with Citrus paradisi(known as Grapefruit) like these are from the same kingdom, order and family and from similar genus. People always miss understand Pomelo as Grape fruit because of their similar outer appearance. There are some differences which can distinguish both of them such as their scientific name, place to grow, health benefits, colour of peel and taste of the pulp. Pomelo is responsible in boosting our immune system, preventing weight gain and as an anti-aging agent while grapefruit aims in controlling the blood sugar level in diabetic patients and helps patient's relief from

insomnia. Fruits juice can be used as a febrifuge, a kind of medicine exert its function in reducing the fever. The seeds of Pomelo play a role in curing the coughs and dyspepsia. Fruits of Pomelo also useful to cure cardio-tonic, in preventing constipation problem, improve digestion and peristalsis**.** Grape fruit is a somewhat bitter fruit. It can be used to lower cholesterol, prevent cancer, aid weight loss, fight skin diseases, relieve constipation, and boost immunity. Fruit juice contains furanocoumarins, including limonin, quercetin, kaempferol and obacunone.

The aim of the present study was to take the regular dietary use of Citrus fruits may provide sufficient prophylaxis. To achieve this aim, undertaken to investigate and compare the antimicrobial and antioxidant activities of fresh juices of *Citrus maxima* (Burm.) Merr.(Pomelo)and *Citrus paradise* Macfad. (Grapefruit).

Materials and Methods

Collection and identification of plant materials

Fresh fruits (Pomelo and Grapefruit) were purchased from the local market in Myitkyina, in the month of August 2018. The mature and medium size of the fruits was used. The fruits were identified by referring the literatures of Hundley and Chit KoKo, 1987 and Kress and Yin Yin Kyi, 2003.

Preparation of fresh juices

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

Antimicrobial activities of fresh juices

Test microorganisms

Five microorganisms were used in this study; two were gram positive bacteria, *Staphylococcus aureus* and *Bacillus cereus* and three were gram negative bacteria *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* by using agar-well diffusion method at the Mandalay Institute of Technology, Department ofBiotechnology, Mandalay.

Determination of antioxidant activity of fresh juicesby DPPH radical scavenging assay

The fresh juices of *Citrus maxima* and *Citrus paradisi*were investigated antioxidant activity by using DPPH (1,1-diphenyl-2-picryl-hydrazyl) Radical Scavenging Assay (Blosis, 1958 and Brand-Williams *et al*., 1995). This assay has

been widely used to investigate the free radical scavenging assay. The tests were conducted at Mandalay Institute of Technology, Department of Biotechnology, Mandalay.

Preparation of test sample solution

Briefly a 2000 µl/ml of DPPH, 1800 µl/ml of aqueous solution was added to 200 µl/ml sample juice solution at different concentration. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance was measured at 517 nm in a spectrophotometer.

Preparation of standard solution

Ascorbic acid (0.001 g) was dissolved in 100 ml of warm water and it was used as a standard solution.

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.

Results and Discussion

Scientific name - *Citrus maxima* (Burm.)Merr.(**Figure1)**

Myanmar name- Kyewkaw

English name - Pomelo

Family - Rutaceae

Figure (1): Pomelofruit & fruit pulp

Scientific name - *Citrus paradisi*Macfad. (**Figure 2)**

Myanmar name- Grapefruit

English name - Grapefruit

Family - Rutaceae


Figure (2): Grapefruit& fruit pulp

Antimicrobial activitiesof fresh juices

The agar-well diffusion method was used to determine the inhibition zone of *Citrusmaxima* and *Citrus paradisi*. The results of the antimicrobial activity were shown in Table 1 and 2.

Pomelo juice showed significant antimicrobial activity against *E. coli* (11 mm) and *Bacillus cereus* was shown (15 mm). *Staphylococcus aureus, Salmonella typhi,* and *Pseudomonas aeruginosa* were not shown any inhibition zone. The results were compared with control (Table 1).

Grapefruit juice showed significant antimicrobial activity against *E. coli, Salmonella typhi* and *Bacillus cereus* were shown 15 mm. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were not shown any inhibition zone. The results were compared with control (Table 2).

By comparison, medium activity was seen in case of *Bacillus cereus* where the inhibition zone was 15 mm on both fresh juices (Table 3).

Table (1): Antimicrobial activities of Pomelo

Test organisms	Inhibition Zone Diameter (mm)	
	Control	Sample
Escherichia coli	0 mm	11 mm
Staphylococcus aureus	0 mm	0 mm
Salmonella typhi	0 mm	0 mm
Pseudomonas aeruginosa	0 mm	0 mm
Bacillus cereus	0 mm	15 mm

Table (2): Antimicrobial activities of Grapefruit

Test organisms	Inhibition Zone Diameter (mm)	
	Control	Sample
Escherichia coli	0 mm	15 mm

Staphylococcus aureus	0 mm	0 mm
Salmonella typhi	0 mm	15 mm
Pseudomonas aeruginosa	0 mm	0 mm
Bacillus cereus	0 mm	15 mm

Table (3): Comparison of antimicrobial activities of Pomeloand Grapefruit

Test organisms	Inhibition Zone Diameter (mm)	
	Citrus maxima	*Citrus parasidi*
Escherichia coli	11 mm	15 mm
Staphylococcus aureus	0 mm	0 mm
Salmonella typhi	0 mm	15 mm
Pseudomonas aeruginosa	0 mm	0 mm
Bacillus cereus	15 mm	15 mm

The results showed that the juices of both fruits exhibited inhibitory actions against the bacterium *E. coli*, *S. typhi* and *B. cereus* were inhibited. Both fruit juices, on the other hand, with regard to diameter of zone of inhibitions, showed appreciable inhibitory potential against *E. coli* and *B. cereus*. Pomelo juice failed to inhibit other three bacteria *S. aureus*, *S. typhi*and *P. aeruginosa* and Grapefruit juices failed to inhibit other two bacteria, *S. aureus* and *P. aeruginosa*.

In this present study an attempt had been made to appraise the antimicrobial potential of some common Citrus fruits against some pathologically important bacteria which might cause serious health hazards in human. *E. coli* is a naturally occurring bacterium in the intestinal tract of man. *S. aureus* is one of the most common bacteria implicated in food poisoning.*P. aeruginosa* may be responsible for pneumonia and diarrhoea etc..*B. cereus* can cause diarrhoea and emetic toxin. *S. typhi*signs and symptoms include nausea, vomiting and diarrhoeacited in Basak *et al.*, 2017.

Determination of antioxidant activity of fresh juices by DPPH radical scavenging assay

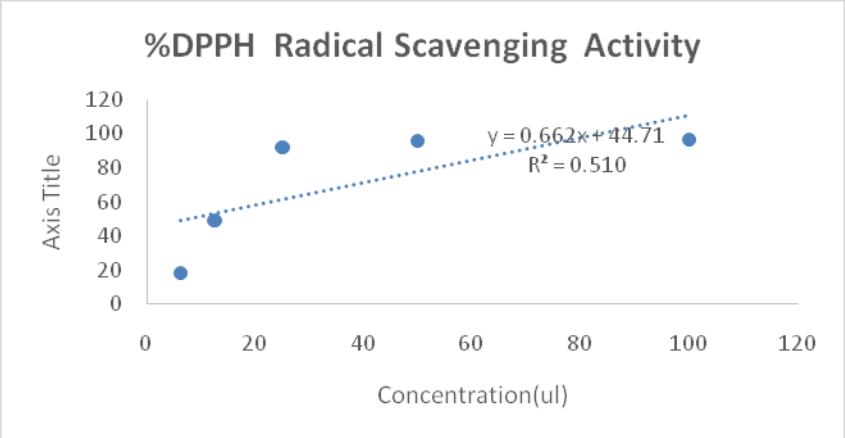
Antioxidant activity of fresh juices and standard ascorbic acid was investigated by the DPPH radical scavenging assay of different concentration. Determination of absorbance was carried out at wave length 517 nm using spectrophotometer. Decrease in absorbance indicates increase in radical scavenging. IC_{50} (50% of inhibition) values were calculated after linear regression analysis of the observed inhibition percentages vs. concentration of sample, where lower IC_{50} values indicate higher antioxidant activity.

According to the result of percentage of radical scavenging activity (% RSA), from fresh juice of Pomelowas shown in Table 4 and Figure 3.IC_{50} value of fresh fruits juice of Pomelois 7.97□l/ml.

Table (4): % Inhibition in various concentrations of Pomelo juice

Sample Concentration (µl/ml)	%DPPH Radical Scavenging Activity	IC_{50} (µl/ml)
6.25	18.14206	7.97
12.5	49.08762	
25	92.0374	
50	96.10918	
100	96.51636	

Figure (3): A graph of % inhibition vs concentration (µl/ml) for standard ascorbic acid

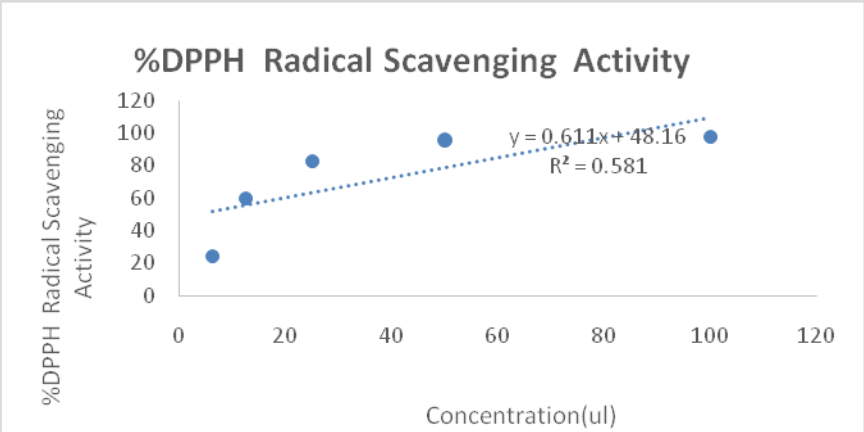


According to the result of percentage of radical scavenging activity (% RSA), from fresh juice of Grapefruit was shown in Table 5 and Figure 4. IC_{50} value of fresh fruits juice of Grapefruitis 3.01□l/ml.

Table (5): % Inhibition in various concentrations of Grapefruit juice

Sample Concentration (µl/ml)	%DPPH Radical Scavenging Activity	IC_{50} (µl/ml)
6.25	24.325139	3.01
12.5	59.553612	
25	82.4008454	
50	95.58136	
100	97.375961	

Figure (4):A graph of % inhibition vs concentration (µl/ml) for standard ascorbic acid



By comparison, the IC_{50} value of fruit juices, lower absorbance of the reaction mixture indicated higher free radical scavenging activity. IC_{50} value of Grapefruit fruit juice showed low value which indicated better antioxidant activity (Table 6).

Ascorbic acid, the most important water soluble antioxidant, was used as a standard antioxidant for comparison purpose. On the basis of absorbance value, % inhibition of samples and standard ascorbic acid were calculated and from which IC_{50} values of standard ascorbic acid was shown in Table 7.

The antioxidant activity of *Citrus maxima* juice showed 15.25 ± 1.08 % inhibitions at aqueous extracts. The petroleum ether extract of *Citrus maxima* showed the highest antioxidant activity 0.14 ± 1.88 % inhibition (Vadivukarasi and Agnesjenitha, 2015). The highest values obtained in *Citrus maxima* peel extract indicated the Pomelo peel exhibited high antioxidant properties (Khan *et al*., 2018). Grapefruit juice exhibited significant antioxidant activity at concentration of 50 and 100 µl/ml and was comparable to standard antioxidant though it contains the least amount of ascorbic acid (Al-Musharifi*et al*., 2015). Oikeh*et al*., 2016 stated that

Grapefruit juice showed 2.60 ± 0.30 % and 24.0 ± 0.10 % inhibition at concentrations of 0.5 and 1.0 mg/ml of extract. The antioxidant activity may be due to the presence of phenolic compounds and/or vitamin C present in the samples.

Table (6): Comparison of % inhibition in various concentrations of fruit juices

Sample Concentration (µl/ml)	Pomelo juice IC_{50} (µl/ml)	Grapefruit juice IC_{50} (µl/ml)
6.25	7.97	3.01
12.5		
25		
50		
100		

Table (7): % Inhibition in various concentrations of ascorbic acid

Sample Concentration (µl/ml)	%DPPH Radical Scavenging Activity	IC_{50} (µg/ml)
0.25	40.89880863	1.13
0.5	43.49268587	
1	47.09696878	
2	52.29980395	
4	70.87920374	
8	76.06695823	
16	83.4866536	

Conclusion

Citrus fruits are cheap and easily available. As revealed by recent study, regular dietary uses of Citrus fruits may provide sufficient prophylaxis against potential infectious agents to the people developing countries including Myanmar where a major portion of people are poverty-stricken and huge burden of medical cost accelerating the poverty further. Based on the antioxidant results, it can be concluded that these fruit juices could be used as a natural source of antioxidant and their regular consumption could provide health benefits to human by shielding against oxidative stress.

Acknowledgements

I would like to express my sincere gratitude to Dr. Saw Sandar Mon, Professor, Dept. of Biotechnology, Mandalay Institute of Technology for her kind permission to use laboratory facilities for this research. I would like to mention my deepest appreciation to Dr. Tun Tun Naing, Lecturer, Dept. of Chemistry, University of Mandalay for his kind suggestions.

References

Abirami, A., Nagarani, G., and Siddhuraju, P.2013. Antimicrobial activity of crude extract of *Citrus hystris* and *Citrus maxima*. IJPSR, Vol. 4, Issue 1.

Al-Musharfi, N.K., Al-WAhaibi, H.S. and Khan, S.A. 2015.Comparison of ascorbic acid, total phenolic content and antioxidant activities of fresh juices of six fruits grown in Oman. J Food Process Technol 6: 513.

Basak, A.K., Chatterjee T., Majumder D., Hussain S.Z., Mallick S. and Chakravarty A. 2017. Evaluation of antimicrobial potential of juices of some common citrus and non-citrus fruits of India. Int. J. Curr. Microbiol. App.Sci:6 (3): 725 – 731.

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.

Cruickshank, R., Duguid J.P., Marmion B.P. and Swain R.H.A. 1975.Medicinal Microbiology. 12th Ed., Vol. II, Printed in Great Britain, Distributed in the USA by Longman Inc. New York.

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

Khan, N.H., Qian C.J., and Perveen N. 2018. Phytochemical screening antimicrobial and antioxidant activity determination of *Citrus maxima* peel. Pharm Pharmacol Int. J: 6(4):279-285.

Kress, W.J., Defilipps R.A., Farr E and Daw Yin YinKyi. 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.

Mehl, F., Marti, G., Boccard, J., Debrus, B., Merle, P., Delort, E. 2014. Differentiation of lemon essential oil based on volatile and non-volatile fractions with various analytical techniques: A metabolomic approach. Food Chemistry, 143, 325–335.

Munwar, S., Roy HarekrishnaandRahaman S. A. 2015.Antioxidant and free radical scavenging activity of *Citrus medica*. Int. J. of Pharma Res. and Health Sci. Vol. 3 (4): 810 – 816.

Oikeh, E.T., Omoregie E.S., Oviasogie F.E. andOriakhik. 2015. Phytochemical, antimicrobial, and antioxidant activities of different citrus juice concentrates. Food science and Nutrition.

Vadivukarasi,G. and Agnesjenitha X.2015.*In vitro* studies on phytochemical analysis and antioxidant activity of *Citrus maxima*.Int.J. of Res in Pharmacology &Pharmcotherapeutics. Vol-4 (2): 245-251.

Zou, Z., Xi, W., Hua. Y., Nie C. and Zhou Z. 2016.Antioxidant activity of Citrus fruits. Food Chemistry 196: 885–896.