Antimicrobial Activity, Antioxidant Activity and GCMS Analysis of Essential Oil from Lemon (Than-Ba-Yo) Peel

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Abstract

The citron (*Citrus limon* L.,) is a small tree, having large fruit. Especially; the essential oil of lemon peel showed fungitoxicity against some fungi. Therefore, the essential oil of lemon peel was extracted by steam distillation method. Then, the essential oil was analyzed by GCMS Autosampler (Trace 1300, ISQ-QD). In GCMS analysis, GC oven temperatures were assigned by four levels in the range of 80 to 280^{o}C. The increasing temperature rates were controlled by 10 to 15 ^{o}C/min; carrier gas, helium at a constant flow 1.0 mL/min. The injector temperature and mass transfer line temperature were fixed at 275 and 280 ^{o}C, respectively. The molecular masses are arranged in 15 to 250 amu (m/z) and assigned Run Time (min) in the range of 2 to 20. According to the GCMS conditions, the essential oil could be deduced as Phellandrene (2.49 min), Pinene (2.96 min), D-limonene (3.48 min), Terpinene (3.78 min) and Citral (6.52 min). The antimicrobial screening of the essential oil was carried out by agar well diffusion method. In the screening, *B. subtilis, S. aureus, P. aeruginosa, B. pumilus, C. albicans* and *E. coli*s pecies were used. From the screening, the antimicrobial activities of essential oil of lemon peel show the high activities with all microorganisms (except *P. aeruginosa*). The antioxidant properties of lemon peel were screened by DPPH method by using UV spectrophotometer. In the screening, ascorbic acid was used as the standard sample. The water and ethanol extract of fresh lemon peel were determined the absorbances and calculated IC_{50} values by linear regression equation. From the study of antioxidant activity, IC_{50} values of standard ascorbic acid, ethanol and water extract were observed as 5.04, 22.36 and 51.23 µg / mL, respectively. According to the experimental results, the lemon peel possesses the effective antioxidant properties.

Keywords; Antioxidant activity, Antimicrobial activity, Essential oil, GCMS analysis, Lemon peel

1. INTRODUCTION

Citron

Citrus limon, lemon, is a small tree in the Rutaceae (Citrus family) that originated in Asia) and is now grown commercially worldwide in tropical, semi-tropical, and warm temperate countries, including the Mediterranean region, for the fruit, which is used fresh and in beverages and cooking, and is also used as a preservative due to its anti-oxidant properties. The lemon tree grows to 6 m (20 ft) tall, and has stout spines. The leaves are dark green, leathery, and evergreen, oblong, elliptical, or oval and up to 14 cm (4 in) long; in contrast to several other citrus species, the petioles (leaf stems) are not winged or only narrowly winged. Flower buds are purplish but flowers open to have 5 white petals, up to 5 cm across. Fruits are globose to oblong, 7.5 to 12.5 cm long, and ripen to yellow, with smooth to bumpy rinds dotted with oil glands. (Bailey and van Wyk. 2012)

Lemon fruits can be highly acidic and are high in citric acid and vitamin C. Their tart flavor is popular in beverages, ice creams and desserts, salad dressings, and many meat and vegetable dishes. Lemons have antioxidant properties, so lemon juice is often added to fresh fruit to prevent oxidation and browning. Lemon peel is used as a flavoring or candied. Lemon oil, obtained from the peel, is used as a wood cleaner and polish, and as a non-toxic pesticide. Traditional medicinal uses for the fruit, peels, oil, and oil obtained from the seeds include treating fever and colic, and as an astringent and diuretic. (Bailey and van Wyk. 2012)

Figure 1. Lemon plant and fruits

Scientific Classification

Kingdom	: Plantae
Order	: Sapindales
Family	: Rutaceae
Genus	: *Citrus*
Species	: *C. limon*
Botanical name	: *Citrus limon* (L.) Osbeck
English name	: Lemon
Myanmar name	: Than-ba-yo

Medicinal Uses

Aromatherapy is a form of alternative medicine in which healing effects are ascribed to the aromatic compounds in essential oils and other plant extracts. Aromatherapy may be useful to induce relaxation but there is not sufficient evidence that essential oils can effectively treat any condition Essential oils should not be interpreted to be cures for chronic disease, or other illnesses. Use of essential oils may cause harm including allergic reactions and skin irritation; there has been at least one case of death. As such, the use of essential oils as an alternative medicine should be approached with caution and skepticism (*Posadzki, et al., 2012)*.

While the lemon or orange are peeled to consume their pulpy and juicysegments, the citron's pulp is dry, containing a small quantity of insipid juice, if any. The main content of a citron fruit is the thick white rind, which adheres to the segments and cannot be separated from them easily. The citron gets halved and depulped, then its rind (the thicker the better) is cut in pieces, cooked in sugar syrup, and used as a spoon sweet, in Greek (*Meena, et al., 2011)*.

From ancient through medieval times, the citron was used mainly for medical purposes: to combat seasickness, pulmonary troubles, intestinal ailments, scurvy and other disorders. The essential oil of the flavedo (the outermost, pigmented layer of rind) was also regarded as an antibiotic. Citron juice with wine was considered an effective antidote to poison, as the ophrastus reported. In the Ayurvedic system of medicine, the juice is still used for treating conditions like nausea, vomiting, and excessive thirst.The juice of the citron has a high Vitamin-C content and is used in the Indian system of medicine as an anthelmintic, appetizer, tonic, in cough, rheumatism, vomiting, flatulence, haemorrhoids, skin diseases and weak eyesight.There is an increasing market for the citron for the soluble fiber (pectin) found in its thick albedo (*Dalia, 2010)*.

The peel essential oil composition from various citron varieties studies showed quantitative differences with regard to the major constituents: limonene, γ-terpinene, geranial and neral.

According to the levels of seven components (limonene, β-pinene, γ-terpinene, neral, geranial, nerol and geraniol), the citron cultivars were classified in four main oil chemotypes (Gabriele, *et al*., 2009).

II. AIM

To apply the essential oil from lemon peel which possesses antimicrobial and antioxidant activities and to reduce the agricultural waste.

III. EXPERIMENTAL

The lemon peel samples were collected from Hlaing Thar Yar Township, Yangon Region. Firstly, the essential oil of fresh lemon peel was extracted by steam distillation method. Then, the extracted essential oil was analyzed by GCMS Autosampler (Trace 1300, ISQ QD, Germany). The antimicrobial activity of extracted essential oil was examined by agar well diffusion method (Mar Mar Nyein, *et al*., 1991). The antioxidant activities of water and ethanol extract from lemon peel were determined by DPPH method.

IV. RESULTS AND DISCUSSION

The Essential Oil of Lemon Peel

The collected fresh lemon peel sample was used to extract the essential oil by steam distillation method.

The peel, extractor and extracted essential oil of citron peel were shown in Figure 2.

Figure 2. Extraction of essential oil from lemon peel by steam distillation

GCMS Analysis of Extracted Essential Oil of Citron Peel

The extracted essential oil of citron peel was analyzed by GCMS Autosampler (Trace 1300, ISQ-QD, Germany) (Figure 2). In GCMS analysis, GC oven temperatures were assigned by four levels in the range of 80 to 280^{o}C. The increasing temperature rates were controlled by 10 to 15 ^{o}C/min; ; carrier gas, helium at a constant flow rate 1.0 mL/min. The injector temperature and mass transfer line temperature were fixed at 275 and 280 ^{o}C, respectively. The molecular masses (mass fragmentations) are arranged in 15 to 250 amu (m/z) and assigned Run time (min) in the range of 2 to 20.

From the GCMS analysis, four peaks from the GC chromatogram were observed at 6.11 and 6.52 min of different run times. The mass fragmentation patterns (m/z values) of each compound were matched with that of reference compounds from GCMS Libraries. By using GCMS, each chromatogram of different run times could be deduced as Phellandrene (2.49 min), Pinene (2.96 min), D-Limonene (3.48 min), Terpinene(3.78 min) and Citral (6.52 min). From the extracted essential oil of lemon peel, Limonene was observed as the highest composition. GCMS analyzed data are shown in Figure 3, 4 (a), (b), (c), (d), (e) and (f).

Figure 3 GCMS Autosampler (Trace 1300, ISQ-QD, Germany)

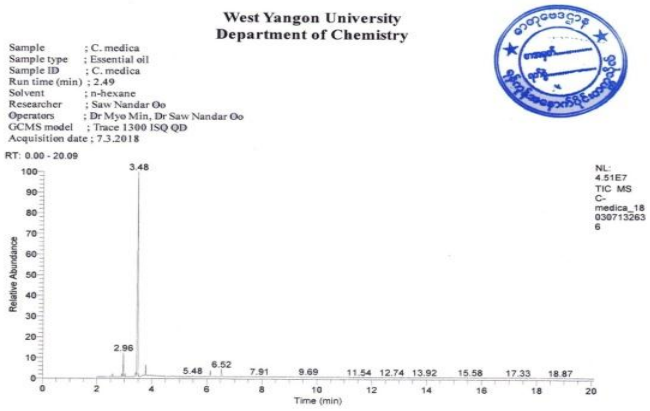

Figure 4 (a) GC chromatogram of the extracted essential oil

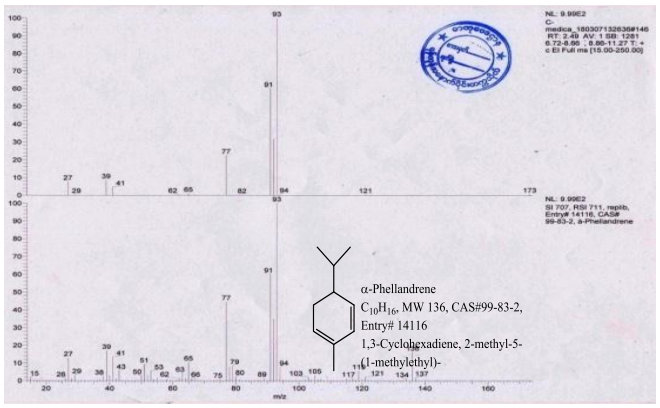

Figure 4 (b) Fragmentation patterns of extracted essential oil (2.49 min) and Phellandrene from GCMS data library

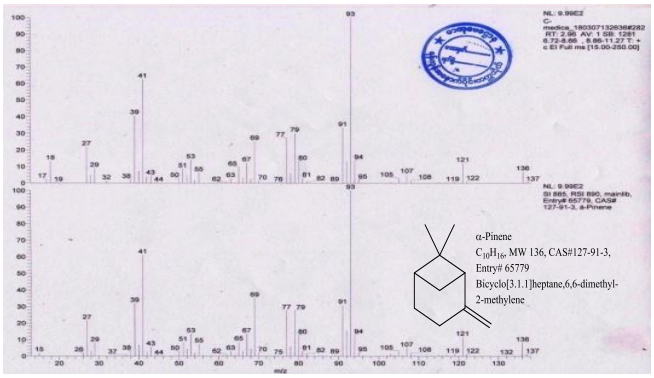

Figure 4 (c) Fragmentation patterns of extracted essential oil (2.96 min) and Pinene from GCMS data library

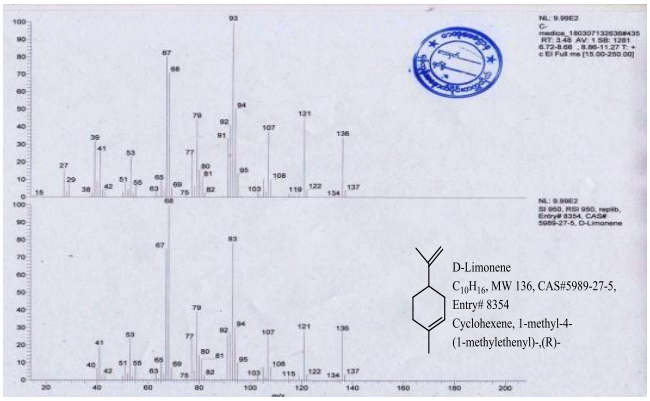

Figure 4 (d) Fragmentation patterns of extracted essential oil (3.48 min) and D-Limonene from GCMS data library

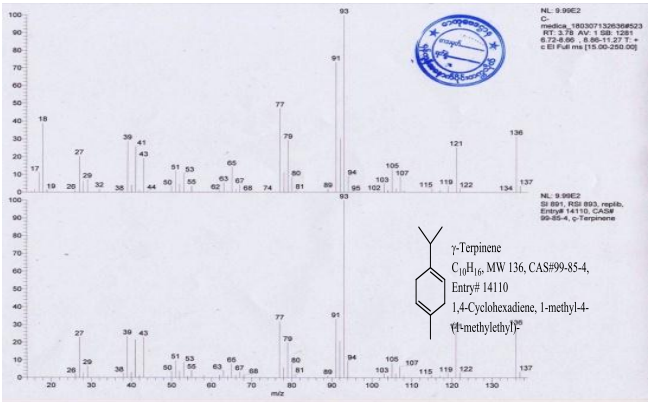

Figure 4 (e) Fragmentation patterns of extracted essential oil (3.78 min) and Terpinene from GCMS data library

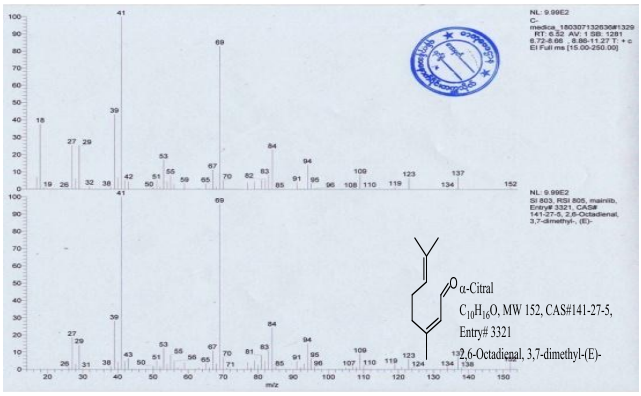

Figure 4 (f) Fragmentation patterns of extracted essential oil (6.52 min) and Citral from GCMS data library

Screening of Antimicrobial activity

The antimicrobial activities of extracted essential oil of lemon peel were screened by agar well diffusion method (Table 1). In this screening, the essential oil was tested on six species of microorganisms; *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans*and *E.coli*species.

In the screening, the antimicrobial activities on all microorganisms (except *P. aeruginosa*) showed the high activities. The activity on *P. aeruginosa*was observed as the medium activity.

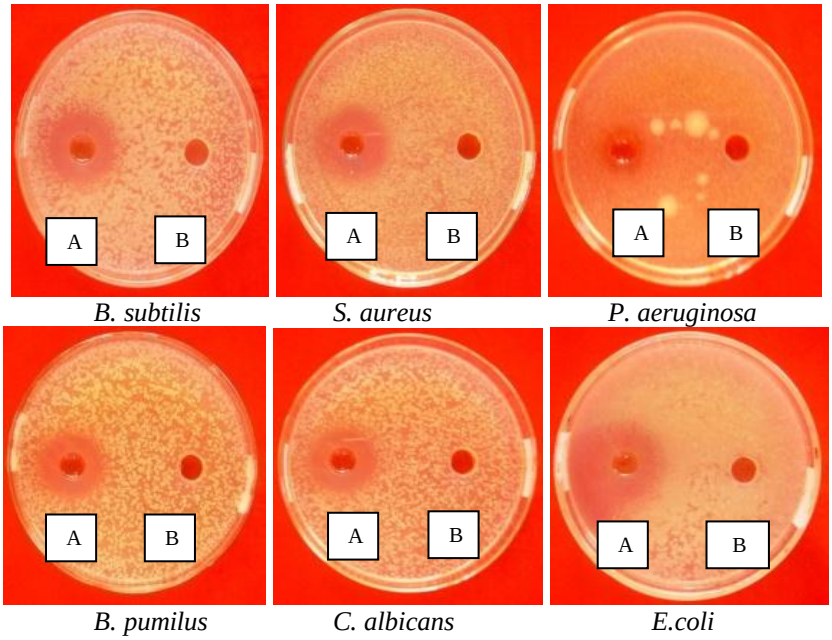

Figure 5. Antimicrobial activities of extracted essential oil

Table 1 Results of Antimicrobial Screening of Extracted Essential Oil of Lemon peel

B. subtilis	***S. aureus***	***P. aeruginosa***	***B. pumilus***	***C. albicans***	***E.coli***
23 mm (+++)	24 mm (+++)	15 mm (++)	23 mm (+++)	23 mm (+++)	26 mm (+++)

Agar well : (10 mm), 10 mm-14 mm (+), 15 mm-19 mm (++), 20 mm-above (+++)

Screening of Antioxidant Activity of Limon Peel by DPPH Method

In the screening of antioxidant activity, water and ethanol extract of lemon peel were determined. Ascorbic acid was used as the standard. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517nm, which is induced by antioxidants. The significant decrease in the concentration of the DPPH radical is due to the scavenging ability of the sample. The different concentrations such as 200µg/mL, 100µg/mL, 50µg/mL, 25 µg/ mL and 12.5µg/ mL were prepared by dilution with ethanol as solvent. The absorbance values were measured at wavelength 517nm for different concentration of water and ethanolic extracts and the control.

From the screening of antioxidant activity, IC_{50} values of standard ascorbic acid, ethanol and water extract were observed with 5.04, 55.96 and 22.36 µg/mL, respectively. Therefore, it can be seen that the orange peel possesses the antioxidant activity. The antioxidant activity of water extract is more potent than that of ethanol extract. The antioxidant activities of standard ascorbic acid and extract samples of orange peel are shown in Table 2, 3, 4, Figure 6, 7, 8 and 9.

Table 2. Absorbance of Different Extracts of Lemon Peel

No.	**Concentration (µg/mL)**	**Ascorbic acid (Standard)**	**Ethanol extract**	**Water extract**
1	12.5	0.220	0.260	0.241
2	25	0.161	0.254	0.173
3	50	0.126	0.141	0.124
4	100	0.059	0.072	0.086
5	200	0.043	0.002	0.043

Table 3. Percent Inhibition of Different Extracts of Lemon Peel

No.	Concentration (µg/mL)	Ascorbic acid (Standard)	Ethanol extract	Water extract
1	12.5	39.89	28.96	34.15
2	25	55.96	30.60	52.84
3	50	65.61	61.48	66.12
4	100	83.88	80.32	76.50
5	200	88.24	99.45	88.14

* Absorbance of DPPH (Control) = 0.366; $\%\ RSA = \frac{Abs_{DPPH} - [Abs_{Sample} - Abs_{Blank}]}{Abs_{DPPH}} \times 100$

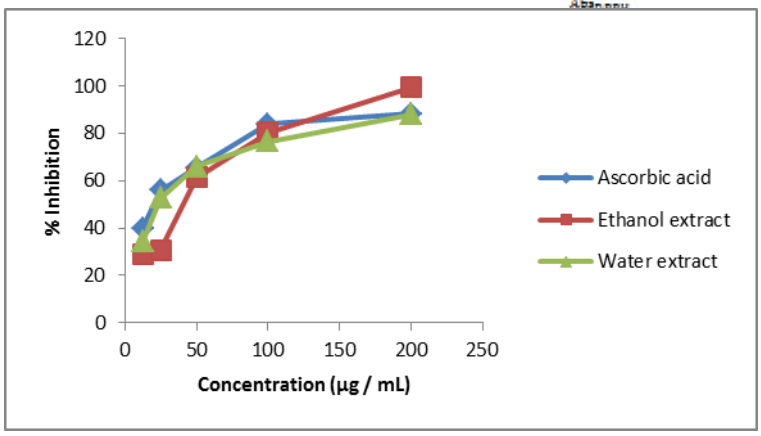

Figure 6. Antioxidant activities of standard ascorbic acid, ethanol and water extract with concentrations

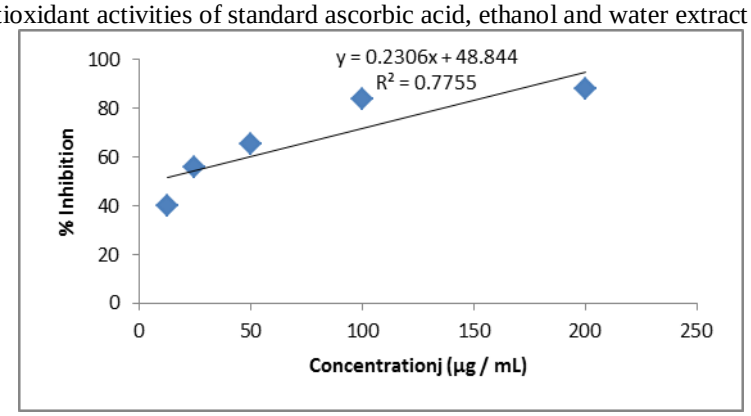

Figure 7. Linear regression equation for antioxidant activity (IC_{50}) of standard ascorbic acid

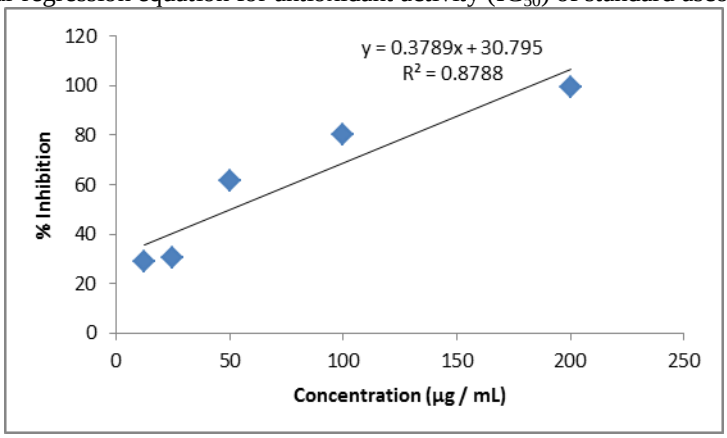

Figure 8. Linear regression equation for antioxidant activity (IC_{50}) of ethanol extract

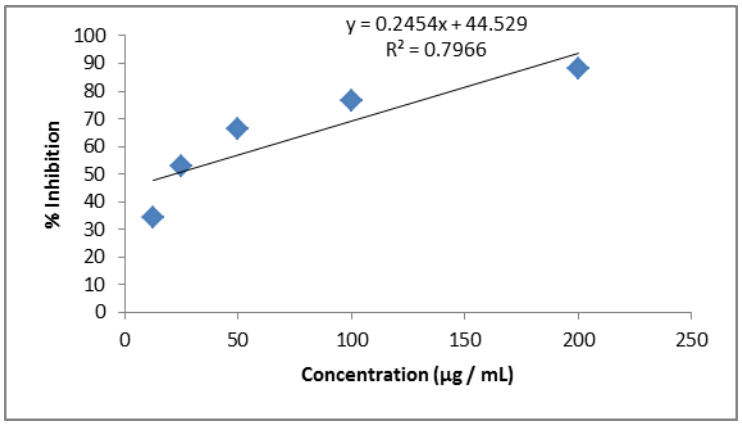

Figure 9. Linear regression equation for antioxidant activity (IC_{50}) of water extract

Table 4 The Regression Equation and IC_{50} Values of Test Samples

No.	Test Solution	Regression Equations	IC_{50} (ppm)
1	Ascorbic Acid	y = 0.230x + 48.84	5.04
2	Ethanol extract	y = 0.378x + 30.79	51.23
3	Water extract	y = 0.245x + 44.52	22.36

V. CONCLUSION

This research concerns with the GCMS analysis and antimicrobial screening of extracted essential oils from the lemon peel. Firstly, the extraction of essential oil from lemon peel was carried out by steam distillation method. Then, the extracted essential oil was analyzed by GCMS method. From the analysis, the essential oils could be deduced as Phellandrene (2.49 min), Pinene (2.96 min), D-Limonene (3.48 min), Terpinene (3.78 min) and Citral (6.52 min). D-Limonene (3.48 min) was observed as the major constituent from the extracted essential oils.

Then, the antimicrobial activity of essential oil from lemon peel was studied by agar well diffusion method. The extracted essential oils were examined with *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *E.coli*species. In the screening, the antimicrobial activities on all microorganisms (except *P. aeruginosa*) showed the high activities. The activity on *P. aeruginosa* was observed as the medium activity. According to the antimicrobial screening, the essential oil of lemon peel may be used in the medicinal formulation of antimicrobial drugs for human health. Especially, due to the activity on *C. albicans*, it may be used in the anti-fungal agents for human skincare.

In the screening of the antioxidant activity of water and ethanolic extract from lemon peel, the results indicate that the lemon peel possess antioxidant properties and exhibited free radical scavenging activity. It was observed that IC_{50} value of standard ascorbic acid was 5.04 µg / mL. The antioxidant activity of water and ethanolic extract were 22.36 and 51.23 µg / mL. According to the comparison of IC_{50} values between standard ascorbic acid and lemon peel extracts, lemon peel possesses the rich antioxidant activity. Therefore, lemon peel may be used in the medicinal antioxidant for human health.

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REFERENCES

Bailey and van Wyk. 2012. "*Citrus limon* (L.) Burm. f.". Flora of Pakistan. Encyclopedia of Life.htm

*Dalia A. A. (2010). "Preparation and Characterization of Pectin from Peel of Kabad (Citrus medica) Fruit in Sulaimani City, Iraqi Kurdistan Region" (PDF). International Journal of Current Research in Chemistry and Pharmaceutical Sciences.**1** (7): 142–146.*

Mar MarNyein.Chit Maung.MyaBwin and Tha, S.J. (1991)."*In Vitro* Testing of Various Indigenous Plant Extracts on Human Pathogenic Bacteria".*Myanmar Health Science Research Journal*.vol**3**. pp. 89-99.

Gabriele B., Fazio A., Dugo P., Costa R. and Mondello L. (2009).Essential oil composition of *Citrus medica* L. cv. Diamante (Diamante citron) determined after using different extraction methods. *J Sep Sci*. **32**:99–108.

*Meena, Ajay K., Kandale A., Rao M. M., Panda P., and Reddy G. (2011). "A review on citron-pharmacognosy, phytochemistry and medicinal uses" (PDF). The Journal of Pharmacy.**2** (1): 14–20.*

*Posadzki P., Alotaibi A. and Ernst E. (2012). "Adverse effects of aromatherapy: A systematic review of case reports and case series". The International journal of risk & safety in medicine.**24** (3): 147–61.*