

Investigation of Chemical Constituents and Bioactivities of Essential Oil Extracted from the Peel of *Citrus limon* Linn. (Than-pa-yo)

Swe Zin Myint¹, San SanHtay², PhyuPhyuLwin³

Abstract

The research paper deals with the investigation of some phytochemicals, nutritional values and antimicrobial activity from peel of *Citrus limon* Linn. (Than-pa-yo). Moreover, isolation of phytochemical constituents of essential oil from peels of *Citrus limon* Linn. is done (Than-pa-yo) by GC-MS method. The preliminary phytochemical tests revealed the presence of alkaloids, α -amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, tannins, steroids and terpenoids whereas starch was absent by test tube method. Nutritional values from peel dried powder of *Citrus limon* Linn. (Than-pa-yo) are protein, ash, moisture, fiber, fat and carbohydrate that were detected by AOAC method. Antimicrobial activity of PE, EtOAc, 95 % EtOH, H₂O extracts and essential oil from peel of *Citrus limon* Linn. (Than-pa-yo) were investigated on six strains of organisms by agar well diffusion method. Ethyl acetate extract and essential oil from peel of *Citrus limon* Linn. (Than-pa-yo) showed antimicrobial activity against all tested microorganisms (13 mm to 26 mm). The essential oil from peel of *Citrus limon* Linn. (Than-pa-yo) was extracted by steam distillation method. The phytochemical components of essential oil were characterized by GC-MS. From the result data, sixteen phytochemical constituents were obtained from essential oil of peel of *Citrus limon* Linn. (Than-pa-yo).

Keywords: *Citrus limon* Linn. (Than-pa-yo), phytochemical tests, nutritional values, antimicrobial activity, essential oil

INTRODUCTION

Herbal medicine contains natural substances that can promote health and reduce illness. There are many herbs, which are used to treat cardiovascular problems, liver disorders, central nervous system, digestive and metabolic disorder. The plant, *Citrus limon* Linn. (Than-pa-yo) invites attention of researcher worldwide for its pharmacological activities. Lemons are a good source of vitamin C and flavonoids or antioxidant. The fruit, juice and peel of Lemon are also used to make medicine. Lemon helps in blood circulation, prevents cholesterol, decrease hypertension, combats arteriosclerosis and heart attack (Sawamura *et al*, 2004). *Citrus limon* Linn. (Than-pa-yo) belonging to family Rutaceae, is a species of small evergreen tree about 11 feet high. It has wide occurrence in the Asia-Pacific region from India, South East Asia and Southern China (Vaioa *et al*, 2010).

The report may be divided into five portions, (i) preparation of different crude extracts and essential oil (ii) investigation of preliminary phytochemicals (iii) determination of nutritional values (iv) screening of antimicrobial activity (v) isolation of phytochemicals constituents from essential oil of *Citrus limon* Linn. (Than-pa-yo).

MATERIALS AND METHODS

Collection and Preparation of sample

Citrus limon Linn. (Than-pa-yo) fruits were collected from Taikkyi Township. After being washed, sample was taken the peel of fruit, and shade dried at room temperature for 15 days. The dried samples were pulverized into fine powder using blender and stored in air tight container to prevent moisture changes and other contamination.

¹Dr, Lecturer, Department of Chemistry, Dagon University

²Dr, Lecturer, Department of Chemistry, Dagon University

³Daw, Lecturer, Department of Chemistry, Dagon University

Preparation of Different Crude Extracts and Essential Oil

Dried powdered sample (100 g) was soaked in 500 mL of petroleum ether (60-80 °C) and kept on a shaker, continuously shaken for about 6 hours and the suspension was allowed to stand for 18 hours. After that, the pet ether extract was filtered under vacuum through Whatman No.1 filter paper and the process repeated until all soluble compounds had been extracted. Extraction was considered to be complete when the filtrate had a faint color. All pet ether extract solutions was recovered by distillation and evaporated to get petroleum ether extract. The residue was percolated in ethyl acetate (500 mL) and carried on above procedure and then ethyl acetate extract was obtained. Then, 500 mL of 95 % ethanol solution was added to the remaining residue to get 95 % ethanol extract according to above procedure. To prepare the watery extract, 10 g of the dried powdered sample was extract with distilled water (200 mL) within 30 min, by using hot extraction. Watery extract solution was evaporated to dryness to give watery extract.

The blending fresh peel sample of *Citrus limon* Linn. (Than-pa-yo) (200 g) and distilled water 500 mL were placed in the round-bottomed flask. The flask was fitted to steam distillation set which was joined to water condenser. When the flask was heated at 80 °C to 90 °C for 3 hours, the mixture of essential oil vapor and water vapor coming out from the condenser were collected in the receiver flask. When the mixture of water and essential oil were nearly removed for 3 hours, the distillation set was turn off. After removing lower distillate water from receiving tank, the residual mixture was transformed into a closed separating funnel. The upper layer of essential oil from the separating funnel was carefully drawn out by a syringe and collected in container.

Preliminary Phytochemical Test

A few grams of dried peels powdered of *Citrus limon* Linn. (Than-Pa-Yo) were subjected to the tests alkaloids, α - amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, starch steroid, tannins and terpenoids according to the standard procedures (Marini-Bettolo *et al*, 1981).

Determination of Nutritional Values

Nutritional values from peel dried powder of *Citrus limon* Linn. (Than-pa-yo) are protein, ash, moisture, fiber, fat and carbohydrate were detected by AOAC method in Food Industry Development Supporting Industry.

Screening of Antimicrobial Activity

Antimicrobial activity of PE, EtOAc, 95 % EtOH, H₂O extracts and essential oil were investigated by agar well diffusion method at the Pharmaceutical Research Department, (PRD), Ministry of Industry, Insein Township, Yangon Region. The microorganisms used in the investigation of antimicrobial activity were *Bacillus subtilis*, *Bacillus pumilus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans*.

Isolation of Phytochemicals Constituents from Essential Oil

GC-MS analysis is an analytical testing method that combines features of gas chromatography and mass spectrometry to identify different substances within a liquid or volatile sample. GC-MS is commonly used to identify unknown samples, contaminants, or residual solvents. GC-MS analysis is an extremely versatile testing method that can also be used for environmental analysis (Mathias, 2015). The mass spectra of phytochemical constituents in essential oil of *Citrus limon* Linn. (Than-pa-yo) were examined by Department of Research and Innovation National Analytical Laboratory (DRI), Yangon.

RESULTS AND DISCUSSIONS

Preliminary Phytochemical Investigation of Peel Sample by Test Tube Method

Phytochemical investigation was carried out to know the types of phytochemical constituents present in selected *Citrus limon* Linn. (Than-pa-yo). According to these results, alkaloids, α -amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, tannins, steroids and terpenoids whereas starch was absent by test tube method. These results are summarized in Table 1.

Nutritional Values of Peel of *Citrus limon* Linn.

Nutritional values from peel dried powder of *Citrus limon* Linn. (Than-pa-yo) are protein (7.72 %), ash (4.5 %), moisture (7.6 %), fiber (15.28 %), fat (2.79 %) and carbohydrate (62.03 %) that were detected by AOAC method. The resulting data was presented in Table 2.

Table 1 Results of Phytochemical Investigation of the Peel of *Citrus limon* Linn. (Than- pa-yo)

No.	Test	Extract	Test Reagent	Observation	Results
1.	Alkaloids	1% HCl	Dragendorff's reagent Mayer's reagent	Orange ppt White ppt	+ +
2.	α -amino acid	H ₂ O	Ninhydrin reagent	Purple spot	+
3.	Carbohydrate	H ₂ O	10 % α -naphthol, H ₂ SO ₄ (conc :)	Red ring	+
4.	Glycosides	H ₂ O	10 % lead acetate	White ppt	+
5.	Phenolic compound	H ₂ O	10 % FeCl ₃	Dark blue	+
6.	Reducing sugar	H ₂ O	Benedict's solution	Green	+
7.	Saponins	H ₂ O	Distilled water	Frothing	+
8.	Starch	H ₂ O	1 % Iodine solution	No deep blue color	-
9.	Tannins	95 % EtOH	5 % FeCl ₃	Green	+
10.	Flavonoids	95 % EtOH	Mg ribbon, HCl (conc :)	Pink	+
11.	Steroids	PE	Acetic anhydride, H ₂ SO ₄ (conc :)	Red color	+
12.	Terpenoids	CHCl ₃	Acetic anhydride, H ₂ SO ₄ (conc :)	Grayish	+

(+) present, (-) absent, ppt = precipitate

Table 2 Results of Nutritional Values from Peel of *Citrus limon* Linn.

(Than-pa-yo)

No.	Parameter	Content (%)
1	Moisture	7.68
2	Ash	4.5
3	Protein	7.72
4	Fiber	15.28
5	Fat	2.79
6	Carbohydrate	62.03

Screening of Antimicrobial Activity by Agar Well Diffusion Method

In this work, antimicrobial activity of various extracts (PE, EtOAc, 95 % EtOH and H₂O) and essential oil obtained from *Citrus limon* Linn. (Than-pa-yo) peel were screened on six different strains of microorganisms such as *Bacillus subtilis*, *Bacillus pumilus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* (*E. coli*) and *Candida albicans* by agar well diffusion method.

The antimicrobial activity of *Citrus limon* Linn. (Than-pa-yo) are shown in Figure 1 & 2 and Table 3. It was found that PE extract, EtOH extract and watery extract did not show activity on all tested microorganisms and only EtOAc extract showed antimicrobial activity (inhibition zone diameter 13-21 mm). Essential oil of *Citrus limon* Linn. (Than-pa-yo) also showed against on six strains. The activities of EtOAc extract on the *Candida albicans* are highest and essential oil on the *Escherichia coli* (*E. coli*) are highest among them. Essential oil was more potent than EtOAc extract.

Table 3 Antimicrobial Activity of Four Crude Extracts and Essential Oil from the Peel of *Citrus limon* Linn. (Than-pa-yo)

Organisms	PE	Inhibition Zone Diameter (mm)			
		EtOAc	95 % EtOH	H ₂ O	Essential Oil
<i>Bacillus subtilis</i>	-	13 (+)	-	-	21 (+++)
<i>Bacillus pumilus</i>	-	18 (++)	-	-	20 (++)
<i>Staphylococcus aureus</i>	-	18 (++)	-	-	21 (+++)
<i>Pseudomonas aeruginosa</i>	-	18 (++)	-	-	20 (++)
<i>Escherichia coli</i>	-	20 (++)	-	-	26 (+++)
<i>Candida albicans</i>	-	21 (+++)	-	-	25 (+++)

Diameter of agar well = 10mm

10 mm ~ 14mm = (+)

15 mm ~ 19 mm = (++)

20 mm above = (+++)

No activity = (-)

Isolation of Phytochemicals Constituents from Essential Oil of *Citrus limon* Linn. (Than-Pa-Yo) by GC-MS Method

The essential oil was extracted from the peel of *Citrus limon* Linn. (Than-pa-yo) and it was analyzed using GC-MS spectroscopy. A total of sixteen phytochemical constituents were obtained from the selected sample. The results are shown in Table 4.

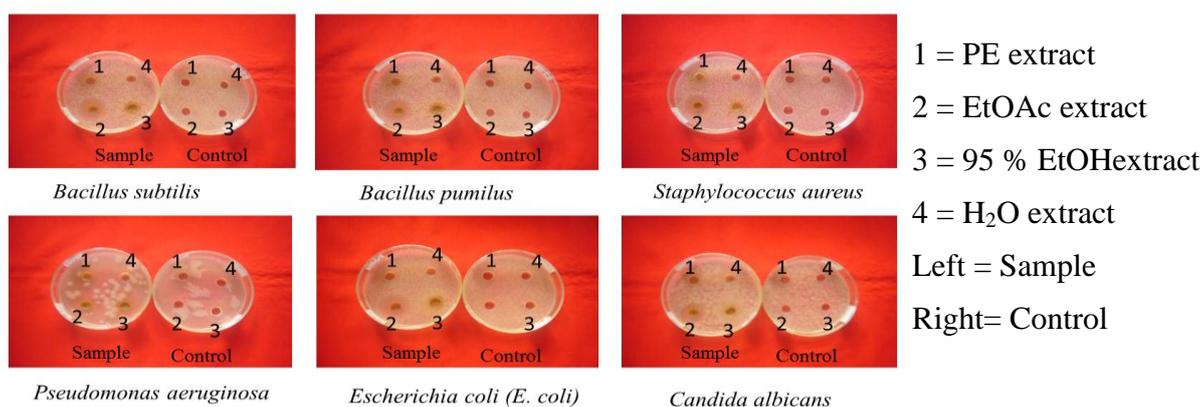
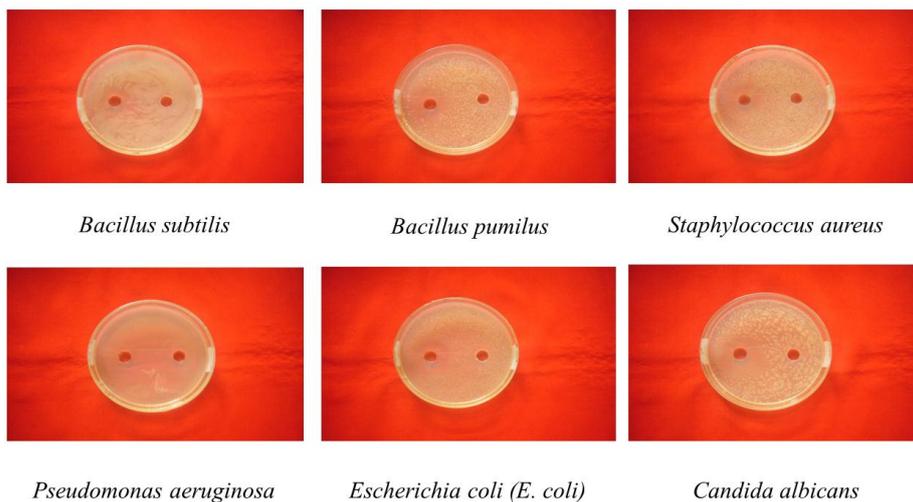


Figure 1 Antimicrobial activity of four crude extracts from the peel of *Citrus limon* Linn. (Than-pa-yo)



1 = Essential Oil 2 = Control

Figure 2 Antimicrobial activity of essential oil from the peel of *Citrus limon* Linn.(Than-pa-yo)

Table 4 Some Phytochemical Constituents of Essential Oil from Peel of *Citruslimon* Linn.(Than-pa-yo)

No.	Phytochemical Constituents
1	Alpha-pinene
2	3-carene
3	D-limonene
4	Beta-ocimene
5	1,6-octadien-3-ol 3,7-diethyl
6	Citronellol
7	Citral
8	Geraniol
9	Trans-carveol
10	Trans-2-undecen-1-ol
11	Caryophyllene
12	Beta-curcumene
13	Beta-bisabolene
14	Alpha-bisabolol
15	Hexadecanal
16	Levomenol

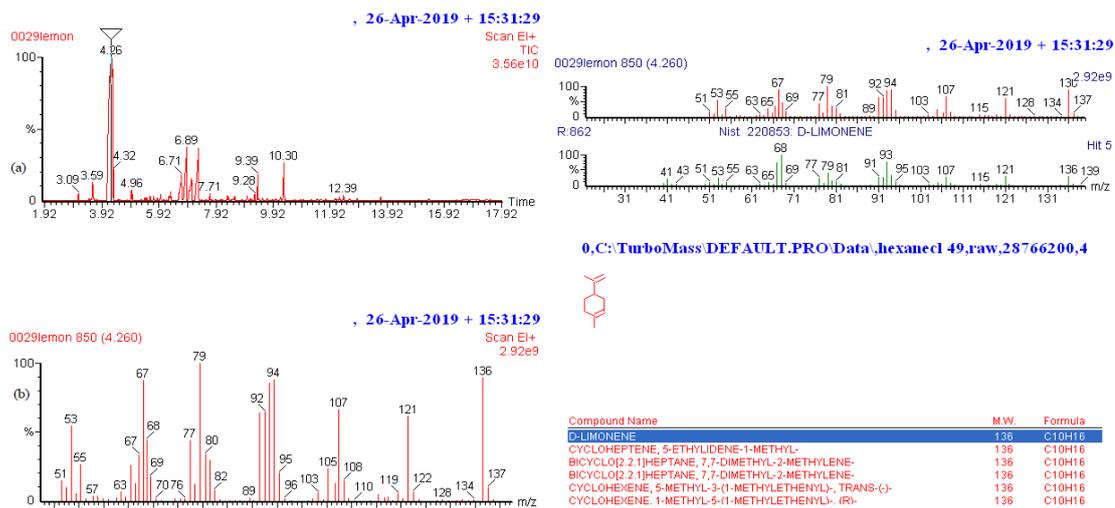


Figure 3 (a) Gas chromatogram and (b) mass spectrum of D-limonene

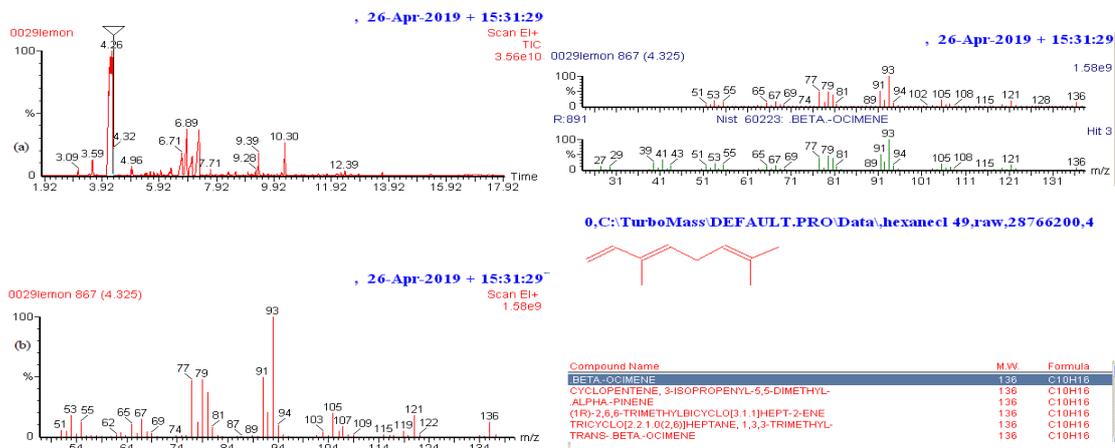


Figure 4 (a) Gas chromatogram and (b) mass spectrum of Beta-ocimene

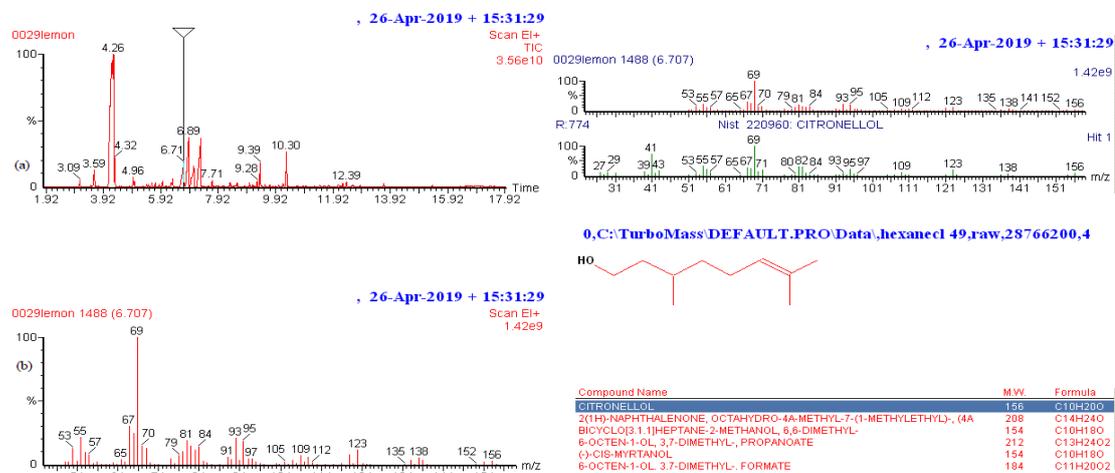
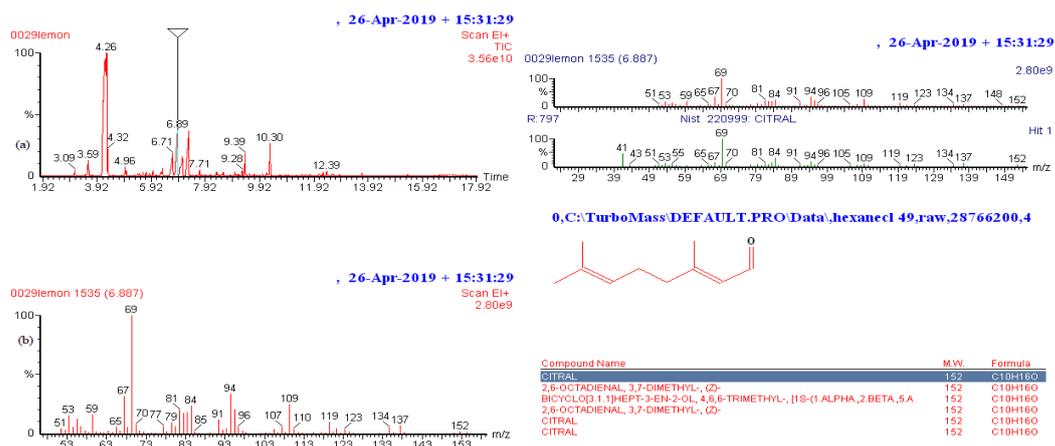


Figure 5 (a) Gas chromatogram and (b) mass spectrum of Citronellol



From the overall assessment of chemical and biological investigation of the selected peel of *Citrus limon* Linn. (Than-pa-yo), it could be concluded as follows:

The preliminary phytochemical tests were performed by test tube method that indicated that the presence of alkaloids, α -amino acids, carbohydrates, glycosides, phenolic compounds, reducing sugars, saponins, tannins, flavonoids, steroids and terpenoids whereas starch was absent. The nutritional values from peel dried powder of *Citrus limon* Linn. (Than-pa-yo) was evaluated by using AOAC method suggested that the sample contained protein (7.72 %), ash (4.5 %), moisture (7.6 %), fiber (15.28 %), fat (2.79 %) and carbohydrate (62.03 %). The yield percent of essential oil was found to 0.48 % base on the fresh *Citrus limon* Linn. (Than-pa-yo) peel sample. The antimicrobial activity of PE, EtOAc, 95 % EtOH, H₂O extracts and essential oil from peel of *Citrus limon* Linn. (Than-pa-yo) was screened by using agar well diffusion method against on six bacteria namely *Bacillus subtilis*, *Bacillus pumilus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans*. In the case of *Candida albicans*, ethyl acetate extract showed highest antimicrobial activity (21 mm). Moreover, essential oil also indicated the highest activity against *Escherichia coli* (26 mm). Therefore, essential oil has more potent antimicrobial activity than ethyl acetate extract. Phytochemical constituents of essential oil from peel of *Citrus limon* Linn. (Than-pa-yo) contained alpha-pinene, 3-carene, D-limonene, beta-ocimene, beta-curcumene, 1, 6-octadien-3-ol 3, 7-diethyl, citronellol, citral, geraniol, trans-carveol, trans-2-undecen-1-ol, caryophyllene, beta-bisabolene, alpha-bisabolol, hexadecanal, and levomenol by GC-MS spectroscopic method.

Therefore, the peel of *Citrus limon* Linn. (Than-pa-yo) may be used for the treatment of diseases caused by bacteria and as natural antioxidant.

ACKNOWLEDGMENTS

We would like to express our profound thanks to Dr. Nu Nu Yee and Dr. Nay Thew Kyi, Pro-Rectors, Dagon University, for their kind permission and encouragement to carry our this work. We are indebted to Dr. Cho Cho Win, Professor and Head, Dr. Khin Than Win, Professor, Department of Chemistry, Dagon University, for their helpful advice, valuable suggestions and provision of research facilities at the Department of Chemistry, Dagon University.

REFERENCES

- AOAC. (2000). "Official Methods of Analysis". *Association of Official Analytical Chemist International*, USA, 7
- Bylka, W., I. Matlawska, and N. A. Pilewska. (2004). "Natural Flavonoids as Anti-Microbial Agents". *Journal of American Natraceutical Association*, **7**(2), 24-31
- Harborne, J. B. (1984). *Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis*, London; 2nd Edition, Chapman and Hall, 120-126
- Marini-Bettolo, G. B., M. Nicolettic and M. Patamia. (1981). "Plant Screening by Chemical and Chromatographic Procedure under Field Conditions". *J. Chromato*, **45**, 121-123
- Mathias, J. (2015). *GC-MS Analysis Deconstructed* "GC-MS Analysis Testing Methods". Inovatech Labs, LLC, 888
- Sawamura, M, H.S. Choi, M. S. L. Kim, N. T. L. Phi., (2004). "Compositional Changes in Commercial Lemon Essential Oil for Aromatherapy". *Journal Aromathera* **14**, 27-36
- Vaioa, C. D., G. Grazianib, A. Gasparib, G. Scaglionea, S. Nocerinoa, and A. Ritieni., (2010). "Essential Oils Content an Antioxidant Properties of Peel Ethanol Extracts in 18 Lemon Cultivars". *Journal of Sciential Horticulturae*, **126**, 50-55
- Vogel, A. I. (1966). *A Text Book of Practical Organic Chemistry*. 3rd Edition, London: Language Book and Longman Group Ltd., 453