



Chemical Composition and Radical Scavenging Activity of *Citrus limon* Peel Essential Oil

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ABSTRACT

The water distilled essential oil of *Citruslimon* collected from Ramsar, Province of Mazandaran, North of Iran collected in December 2013, was analyzed using gas chromatography (GC) and gas chromatography-mass spectroscopy (GC-MS). The yield of oil was 0.23% w/w. Twenty-one components representing 100% of the essential oil were characterized. Limonene (61.4%), b-pinene (13.1%) and g-terpinene (11.3%) were identified as the main constituents in the volatile oil. The antioxidant ability of the oil was examined by free radical scavenging method using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical at different concentration of the oil. The *Citruslimon* oil exhibited free-radical-scavenging properties with IC50 value of 284.71 $\mu\text{g ml}^{-1}$.

Keywords: *Citrus limon*, Essential oil, Radical Scavenging, DPPH.

INTRODUCTION

Essential oils are complex mixture of volatile compounds which consist of terpenes and their oxygenated derivatives such as aldehydes, alcohols and ketones with high potential bioactivity like antibacterial, antifungal and antioxidant that extracted mainly using steam and hydrodistillation^{1,2}.

The genus *Citrus* which belonged to Rutaceae family is represented in Iran by several species such as *C. sinensis*, *C. medica*, *C. limon*, *C. nobelis*, *C. aurantifolia* and *C. aurantium*. *Citrus*

fruits are the most common subtropical plants in the world with a numerous variation due to frequent bud mutation, interspecific and intergeneric hybridization. In Iran, the phylogeny of many citrus variants is remained unknown³. In last decade, the essential oil of *Citrus limon* were the subject of previous study and limonene, a hydrocarbon monoterpene, is dominant constituents which shown sufficient antifungal activity⁴. The oil of *C. limon* was shown acceptable activity against *Anopheles stephensi* (malaria agent) compare to N, Ndiethyl-3-methylbenzamide (Deet) as a standard synthetic repellent⁵.

This study deals with the composition characterization and antioxidant activity using free radical scavenging method of *Citrus limon* essential oil with cultivated in the North of Iran for the first time.

MATERIAL AND METHODS

Plant Material

The fruit of *Citruslimon* were harvested in from Ramsar, province of Mazandaran, North of Iran in December 2013. The collected material was identified in the Citrus Research Institute of Iran (Ramsar, Mazandaran).

Essential Oil Extraction

The *Citruslimon* fruits were washed with cold water and peeled. The peels of fruits were dried in shade and grinded. 100 gr of grinded peels were subjected to hydrodistillation using a Clevenger type apparatus for 3 h. The pale yellowish oil was dried over anhydrous Na_2SO_4 and kept in 4°C until analysis⁶.

Table1: Chemical composition of *Citrus limon* essential oil

Compounds	KI	(%)
Nonane	900	0.1
a-thujene	930	0.6
a-pinene	939	2.4
sabinene	975	2.3
b-pinene	979	13.1
myrcene	991	1.6
a-terpinene	1017	0.3
limonene	1029	61.4
z-b-ocimene	1037	0.3
E-b-ocimene	1050	0.2
g-trpinene	1060	11.3
terpinolene	1089	0.6
a-terpineol	1188	0.4
neral	1238	1.1
geranial	1267	1.5
neryl acetate	1362	0.5
geranyl acetate	1381	0.3
b-caryophyllene	1419	0.4
E-a- bergamotene	1435	0.6
bicyclogermacrene	1500	0.2
b-bisabolene	1506	0.8

Qualitative analysis of *C. limon* oil components

The qualitative analysis was done using a Hewlett-Packard 6890 GC coupled to a Hewlett-Packard 5973 mass selective detector equipped with a HP-5MS (30m × 0.25 mm, 0.32 μm film thickness) column. Oven temperature was programmed from 60°C (3 min) to 230°C at 6°C/min, and the final temperature kept for 3 min. split (1:30) injector temperature was 250°C. Carrier gas was He (99.999%) at 1 ml/min flow rate. The volume of injected sample was 1.0μl of diluted oil in hexane. The mass spectra were achieved at ionization energy 70eV, in the electronic ionization (EI) mode. Ion source temperature was 230°C. Scan mass range was adjusted of m/z 40-650.

The constituents of essential oil were characterized based on their similarity of their mass spectra with those gathered in the Wiley library, or reported in the literature and their relative retention Index (RRI), calculated in relation to the retention time of a series of alkanes (C_7 - C_{20}) as reference chemicals, in comparison with those of the chemical compounds gathered by Adams data⁷.

Quantitative analysis of *C. limon* oil components

The isolated oil was dissolved in n-hexane, and 1.0μl was injected to a Hewlett-Packard 6890

Table 2: %RSA and absorbance of different concentration of *Citrus limon* essential oil

Oil concentration (μg ml ⁻¹)	absorbance	%RSA
DPPH [·] (blank)	1.35	-
1	0.93	31.11
5	0.92	31.85
10	0.91	32.59
50	0.8	40.74
100	0.81	40
200	0.76	43.7
400	0.61	54.81
600	0.47	65.18
800	0.26	80.74
1000	0.01	99.26
Positive control		
Ascorbic acid (100 μg ml ⁻¹)	0.64	37.6

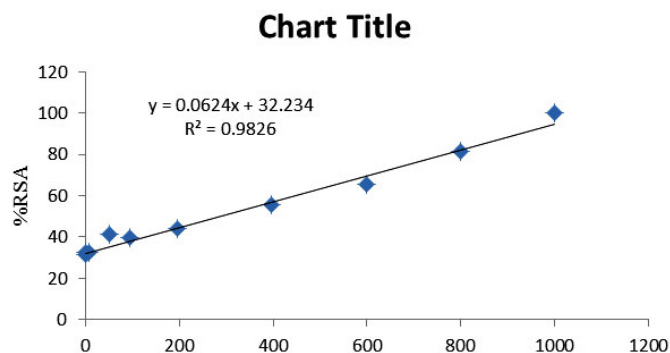


Fig. 1: Relation of *Citrus limon* essential oil concentration and %RSA

gas chromatograph equipped with HP-5 capillary column (30 m × 0.25 mm, film thickness 0.32 µm). The operating conditions were as follows: oven temperature program from 60°C (3 min) to 230°C at 6°C/min heating rate, kept for 3 min at the final temperature, split injection ratio 1:30, carrier gas nitrogen, flow rate 1 mL/min, temperature of injector and detector (FID) fixed at 260°C and 280°C, respectively.

Antioxidant activity of *C. limon* essential oil

The stable organic radical DPPH has been widely used in the determination of the antioxidant activity of different plant extracts. This procedure is based on the reduction of DPPH solutions in the presence of plant extract. DPPH solutions show a strong absorption band at 517 nm appearing a deep violet color. The ability of *C. limon* essential oil to quench reactive species by hydrogen (H⁺ ions) donation was measured through DPPH radical scavenging activity assay. Activity was measured as relative decrease absorbance at 517 nm as reaction between DPPH and the oil. Antioxidant activity was evaluated with %50 (IC₅₀)⁸. A 2 ml of 0.1 mM DPPH methanol solution with 2 ml sample with 1, 5, 10, 50, 100, 200, 400, 600, 800 and 1000 µg ml⁻¹ concentration with shaking. After the solution was incubated for 30 min at 25°C in dark, the decrease in the absorbance at 517 nm was measured. Control contained methanol instead of sample solution. The radical scavenging activity percentage (%RSA) was calculated by the following equation. Ascorbic acid (100 µg ml⁻¹) was used as positive control.

$$\%RSA = \left[\frac{OD_{DPPH} - OD_{sample}}{OD_{DPPH}} \right] \times 100$$

RESULT AND DISCUSSIONS

The pale yellowish essential oil of *Citrus limon* was obtained in the yield of 0.23 %w/w. The chemical composition of *Citrus limon* essential oil was listed in Table 1. Twenty one components, representing 100% of the total oil, were identified in *Citrus limon* essential oil. Limonene (61.4%), b-pinene (13.1%) and g-terpinene (11.3%) were the main constituents. The oil contained 94.1% hydrocarbon monoterpenes, 3.8% oxygenated monoterpenes, 2.0% hydrocarbon sesquiterpenes and 0.1% non-terpenes compounds.

DPPH radical scavenging activity

DPPH radical-scavenging activity percentage (%RSA) was reported in Table 2. The values of DPPH %RSA were presented in table 2 and the relation of extract concentration and %RSA was presented in figure 1.

As shown in figure 3, the IC₅₀ concentration could be calculated using equation of curve ($y = 0.0624x + 32.234$, $R^2 = 0.9826$) by replacing the amount of 50 instead of Y. The *Citrus limon* showed the IC₅₀ at 284.71 µg ml⁻¹. The amount of %RSA for the concentration of 100 µg ml⁻¹ of essential oil and ascorbic acid (40.00 and 37.6%, respectively) were showed close free radical scavenging activity.

CONCLUSION

Demands for natural substance are increasing by food, cosmetic and medicine industry due to consumer's needs. So the importance of studies on essential oils lies not only in the

identification of their constituents but also in the possibility of linking the chemical contents with particular bioactive functional properties. The capacity of essential oils to prevent disease is an interest of researchers. There is a strong need to understand the preventive effect of essential oils for counteracting oxidative damages. Our study suggested that essential oil of *Citrus limon* peel can be considered as an auxiliary supplement.

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