

## Characterization of Antioxidant Property and Chemical Composition of Lemon (*Citrus lemon L.*) Peel Extracts

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### Abstract

Natural products provide opportunities for new drug uses and discoveries because of the unmatched availability of chemical diversity. The use of medicinal plants as natural antimicrobial agents is gaining popularity. Lemon (*Citrus lemon L.*) peels have important constituents, which can be used for pharmacological or pharmaceutical purposes. The lemon peels were extracted with different solvent system, including cold water, hot water, ethanol, methanol and ethyl acetate. All of the extracts tested against antibiotics Ciprofloxacin (10 mcg), Gentamicin (10 mcg) and Rifampin (5 mcg) as control. The bacterial activity of all extracts evaluated against three pathogens (*Escherichia coli*, *Klebsiella* and *Pseudomonas*), using disc diffusion method and broth dilution method. All extracts of Lemon peel were found to be effective against selected bacterial pathogens, in particular, methanol 90% extract showed maximum zone of inhibition (ZOI) of 22 mm. However cold water and ethyl acetate extracts showed minimum effective against *Escherichia coli* pathogens, the zone of inhibition was 8 mm and 7 mm respectively. Gas Chromatography (GC) analysis of the extracts determined the presence of biologically active compounds in ethanol, methanol and ethyl acetate extracts.

**Keywords:** lemon peels; antibacterial; ZOI; antibiotics; GC.

## Introduction

Fruits are generally consumed for their nutritive value and bioactive compounds [1]. Fruits have become the main subject for researchers to investigate since their bioactive compounds are closely related with herbs, commonly referred as phytochemicals such as carotenoids, polyphenols and flavonoids that are abundantly present in fruits and vegetables [2, 3]. Furthermore, natural compounds in fruits and vegetables have shown very promising results against bacteria, fungus and viruses [4, 5].

Lemon (*Citrus lemon L.*) is an important medicinal plant of the family *Rutaceae* [6]. It is cultivated mainly due to its unique taste, shape and the flesh colour [4, 7]. They are rich of phenolic compounds [4, 7, 8, 9, 10]. The *Citrus* peel is a rich source of flavonoid glycosides [11, 12, 13, 14], coumarins [15, 16, 17, 18, 19], sitosterol, and volatile oils [20, 21, 22, 23]. Citrus contents have a large spectrum of biological activity including [1, 2, 8, 9, 10] antioxidant [2, 4, 5, 7], antimicrobial [24, 25], antibacterial [22], antifungal [26], and anticancer [13]. In plants, they appear to play a defensive role against invading pathogens, including bacteria, fungi and viruses [18, 27, 28]. The fiber of citrus fruit contains bioactive compounds, [21, 22] the most important one is ascorbic acid (vitamin C), and it certainly prevents and cures vitamin C deficiency [29, 30]. The studies showed that lemon peels are effective toward various bacteria due to its chemical contents [3, 5, 11, 14, 23]. However, investigations on the biological activities of the fruit peels are lacking due to less popular in commercial application [2]. Biological activities of the peel extract are directly related with the components. The present study was aimed to evaluate the antibacterial properties of lemon peel extracts, different solvent systems will be used including; cold and hot water, ethanol, methanol and ethyl acetate. GC will be used to determine the chemical composition of the extracts.

## Material and Methods

### Materials

Fresh and healthy fruits of Lemon (*Citrus lemon L.*), were collected from Rania, Kurdistan region - Iraq. Fruits were washed and sterilized with distilled water (DW), air dried and packed in envelopes for drying in hot air oven at 50°C for 5 days, until constant weight of the samples.

### Preparation of Extracts

Dried peels were ground into fine powders by the help of grinders. 30g of powdered peels was extracted in 100 ml of solvents for 48 hours in a conical flask (250 ml) at <40 °C. Different solvent systems were used (Table 1). The extracts were filtrated and they were condensed by rotary evaporator (Buchi R-114 Rotavapor, Switzerland) with water bath, temperature set to 40 °C, until all solvent evaporated and solid fine powder obtained.

Table 1. Solvent system that used for lemon peel extraction

Solvents	Percentages of solvent (%)
Ethanol (V/V)	40%, 60% and 90%
Methanol (V/V)	40%, 60% and 90%
Ethyl acetate (V/V)	40%, 60% and 90%
Hot water	100%
Cold water	100%

The crude extracts were freeze-dried to due date for the examination. Dried crude extracts were prepared in a concentration of 2 mg/ml, by dissolving the extracts in sterile distilled water. However for cold and hot water extraction 5g of extracts were used for 72 hours. After that the solutions were filtered through Whatman Qualitative Filter Papers (No. 1, 25 µ; Sigma-Aldrich – UK).

#### **Bacterial Strains and Culture Preparation**

The Bactria species including *Escherichia coli*, *Klebsiella* and *Pseudomonas* were used. With some modification of extraction and isolation procedures of Baron et al. (1994) was used to extraction and monitor the biological activity. Bacterial strains were maintained on nutrient agar plates. They were sub-cultured weekly and subsequently stored at 4°C. The strains was inoculated in the nutrient broth (pH 7.0) and incubated at 37 °C for 24 hours.

#### **Studying of Antibacterial Activity**

To determine the growth of zones of inhibition (ZOI) for bacterial growth Agar diffusion technique was used, However of the determination of the minimum inhibitory concentrations (MICs) Agar dilution technique, was used. Mackonki and Niutrant Agar were used as media culture with different extracted solution. Ciprofloxacin, Gentamicin and Rifampin used as control.

#### **Antimicrobial Test**

For screening of antimicrobial activity of extract plates antimicrobial susceptibility assay was performed by agar well diffusion method to compare their effectiveness against antimicrobial activity, also called cup plate method (Kirby Bauer method).

#### **Antibacterial Properties**

To determine the antibacterial spectrum of the extract, antibacterial susceptibility assay was performed by the agar well diffusion assay, also called cup plate method (Kirby Bauer method). Sterile Mackonki and Niutrant Agar media was prepared and was poured into sterile petri dish plates and allowed to solidify. 30 µl of bacterial pathogens were spread on respective plates labelled earlier as *Escherichia coli*, *Klebsiella* and *Pseudomonas*. Three wells of 8 mm diameter were bored using a sterile cup-borer. 25 µl of Ampicillin (2 mg/ml), crude antimicrobial extract and autoclaved distilled water were poured into the respective wells and the plates were

incubated at 37 °C overnight. The antibacterial activity of each extracts were expressed in terms of the mean of zone diameter of inhibition (in mm) produced by each extracts at the end of incubation period.

### **GC Analysis**

The extracts of 90% of ethanol, methanol and ethyl acetate subjected to GC. Identification for the compounds was done by comparing between the compounds peaks with standards and reference library. GC of a Thermofisher Trace 1300 used, GC fitted with a DB-5 column (L 30 m, ID 0.25 mm, DF 0.25 mm). Injector type is a PTV, with an injection volume of 1 µl at 250 °C was used with a split flow of 20 ml min<sup>-1</sup>. The initial temperature was set at 150 °C, which was held for 1 minute. The temperature of oven was increased at a rate of 10 °C/min until final temperature, which was 260 °C. The detector was a Thermofisher ITQ900. Electron impact (70 eV) ionisation was used for fragmentation with rate of 2 scans per second. All processing and analysis was carried out on Thermo Xcalibur, v 2.2.

### **Results and Discussion**

The dried lemon peel were extracted using different solvent systems with different polarity, each solvent would give an extract. The extracts were subjected to antibacterial susceptibility assay by agar well diffusion method.

Cold water extracts of lemon peels were subjected to antibacterial susceptibility assay by agar well diffusion method and the results shown in Table 2. The extract and antibiotics used against *Escherichia coli*, *Klebsiella* and *Pseudomonas* as pathogens to test the antibacterial activity. The ZOI of cold water for *Escherichia coli* was 8 mm and 4 mm for *Klebsiella* but has no response toward *Pseudomonas* species. In addition, Ciprofloxacin, Gentamicin and Rifampin were used as antibiotics against aforementioned pathogens. The antibiotics showed response only against *Escherichia coli*, but for *Klebsiella* and *Pseudomonas* were inactive. Distilled water was used against the pathogens, but in assays showed no activity.

Hot water extracts of lemon peel were subjected to antibacterial susceptibility assay by agar well diffusion method (Table 2). Hot water extract was highly active against the pathogens compare to cold water, the ZOI against *Escherichia coli* was 15 mm, while by cold water was 8 mm. The ZOI of hot water extract was 6 mm against *Klebsiella*, however cold water was 4 mm. Both water extract were inactive against *pseudomonas*.

Table 2. Antibacterial susceptibility assay of cold water extract of lemon peels

No	Pathogens	ZOI of cold water extracts in mm	ZOI of hot water extracts in mm	ZOI of Ciprofloxacin (10 mcg) in mm	ZOI of Gentamicin (10 mcg) in mm	ZOI of Rifampin (5 mcg) in mm	ZOI of DW in mm
1	Escherichia coli	8	15	9	7	7	0
2	Klebsiella	4	6	0	0	0	0
3	Pseudomonas	0	0	0	0	0	0

Ethanol extracts of lemon peel were subjected to antibacterial susceptibility assay by agar well diffusion method, shown in Table 3. Three fraction of ethanol as solvent were used, 40%, 60% and 90%. As ethanol percentages increased the activity of the extract increased and the ZOI increased as well. ZOI of ethanol extracts 40%, 60% and 90% against *Escherichia coli* were 7, 8 and 18 mm respectively, also ZOI against *Klebsiella* were 4, 6 and 10 mm respectively. Ethanol extracts had no activity against *Pseudomonas*.

Table 3. Antibacterial susceptibility assay of ethanol extract of lemon peels

No.	Pathogens	ZOI by ethanol extracts in mm		
		40%	60%	90%
1	<i>Escherichia coli</i>	7	8	18
2	<i>Klebsiella</i>	4	6	10
3	<i>Pseudomonas</i>	0	0	0

The 90% ethanol extract was dried, and then redissolved in methanol. The extract subjected to GC (see Figure 1) because the extracts had the higher ZOI compare to other ethanol extracts. The Chromatogram showed that the extract contains 7 compounds. The compounds were Coumarin, Ascorbic acid, Citric acid, Linoleic acid (C18:2), Limonoid and Malic acid. Linoleic acid (C18:2) is polyunsaturated fatty acid, while the other compounds are phenolics and flavonoids, they are biologically active [6].

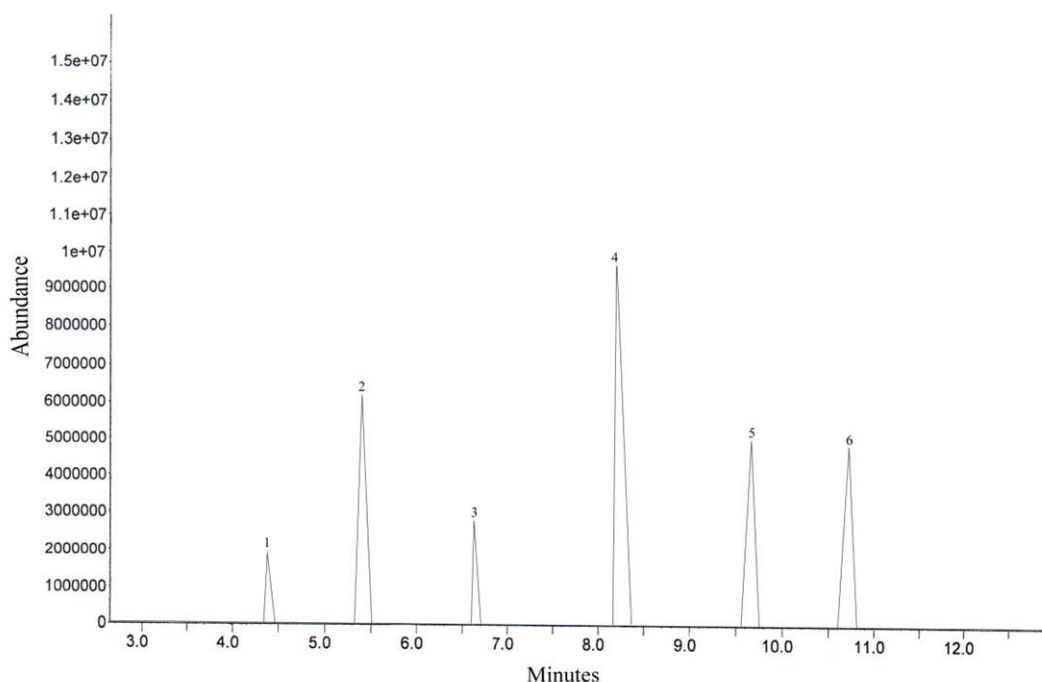


Figure 1. A GC Chromatogram of ethanol extracts, 1: Coumarin, 2: Ascorbic acid, 3: Citric acid, 4: Linoleic acid (C18:2), 5: Limonoid and 6: Malic acid.

Methanol extracts of lemon peel were subjected to antibacterial susceptibility assay by agar well diffusion method and the results are presented in Table 4. The methanol extracts showed activity against pathogens like ethanol extracts. ZOI of methanol extracts 40%, 60% and 90% against *Escherichia coli* were 8, 14 and 22 mm respectively, also ZOI against *Klebsiella* were 6, 8 and 12 mm respectively. Overall, the ZOI of methanol extracts were higher than the ethanol extracts and water extracts. Similar to other extracts methanol extracts had no activity against *Pseudomonas*.

Table 4. Antibacterial susceptibility assay of methanol extract of lemon peels

No.	Pathogens	ZOI by methanol extracts in mm		
		40%	60%	90%
1	<i>Escherichia coli</i>	8	14	22
2	<i>Klebsiella</i>	6	8	12
3	<i>Pseudomonas</i>	0	0	0

The 90% methanol extract was dried, and then redissolved in methanol. The extract subjected to GC because the extracts had the higher ZOI compare to other methanol extracts. The GC chromatogram of methanol extract is shown in figure 2 The Chromatogram showed that the extract contain 7 compounds. The compounds were Coumarin, Ascorbic acid, d-limonene, Linolenic acid (C18:2), Limonoid, Malic acid and B-carotene. Linoleic acid (C18:2) is polyunsaturated fatty acid, while the other compounds are phenolics and flavonoids, they are biologically active.

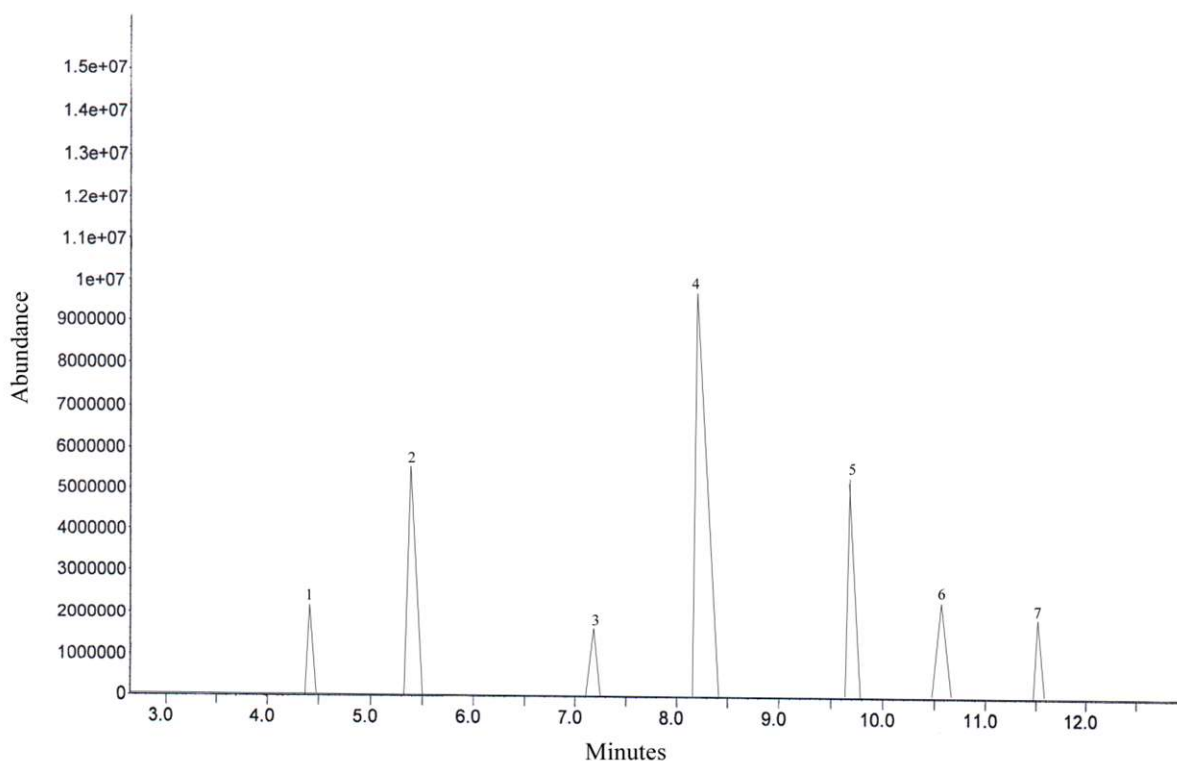


Figure 2. A GC Chromatogram of methanol extracts, 1: Coumarin, 2: Ascorbic acid, 3: d-limonene, 4: Linoleic acid (C18:2), 5: Limonoid, 6: Malic acid and 7: B-carotene. Ethyl acetate extracts of lemon peel were subjected to antibacterial susceptibility assay by agar well diffusion method (Table 5). ZOI of 40%, 60% and 90% ethyl acetate extracts against *Escherichia coli* were 2, 4 and 7 mm respectively. But only 40% ethyl acetate extracts showed activity against *Klebsiella*, which was 6 mm. Similar to other extracts ethyl acetate extracts had no activity against *Pseudomonas*.

Table 5. Antibacterial susceptibility assay of ethyl acetate extract of lemon peels

No.	Pathogens	ZOI by ethyl acetate extracts in mm		
		40%	60%	90%
1	<i>Escherichia coli</i>	2	4	7
2	<i>Klebsiella</i>	6	0	0
3	<i>Pseudomonas</i>	0	0	0

The 90% ethyl acetate extract was dried, and then redissolved in methanol. The extract subjected to GC because the extracts had the higher ZOI compare to other ethyl acetate extracts (Figure 3). The Chromatogram showed that the extract contain 5 compounds. The compounds were Coumarin, Ascorbic acid, d-limonene, Linolenic acid (C18:2), Limonoid, Malic acid and B-carotene. Linoleic acid (C18:2) is polyunsaturated fatty acid, while the other compounds are phenolics and flavonoids, they are biologically active.

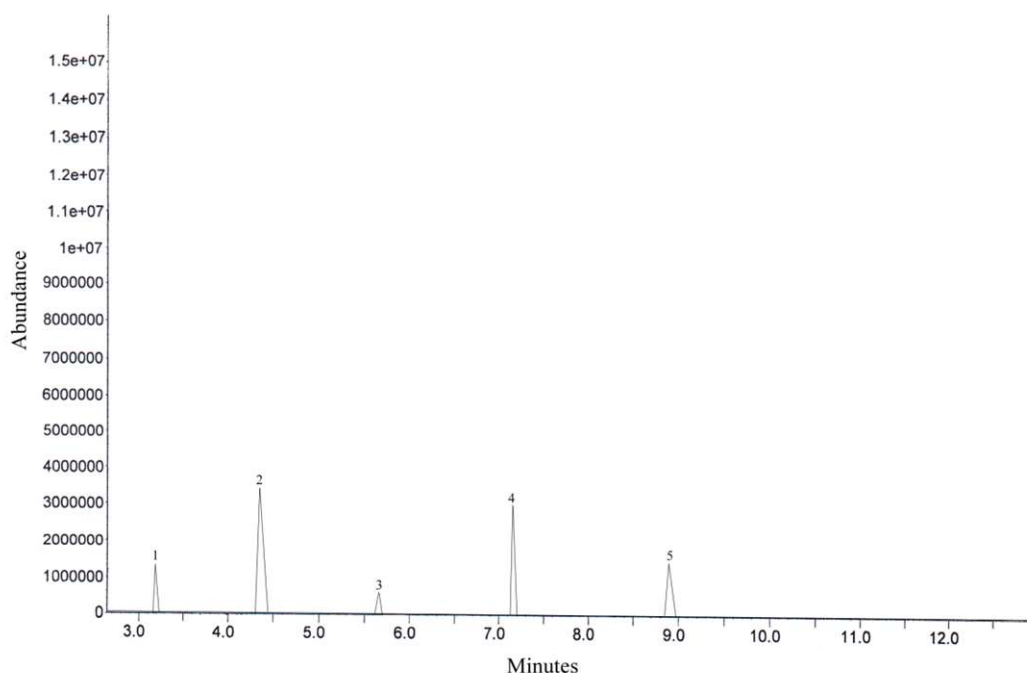


Figure 3. A GC Chromatogram of ethyl acetate extracts, 1:Niacin, 2: Coumarin, 3:d-limonene, 4: Thiamin and 5:linalool.

### Conclusion

Lemon (*Citrus lemon L.*) has a chemical content with potential uses. All parts of lemon reported to have a biological activity, as antibacterial and antimicrobial. The peel as a part is mostly discarded during food consumptions. The lemon peels were extracted with different solvent system, and then the extracts were tested as antibacterial agent against three bacterial species, which are *Escherichia coli*, *Klebsiella* and *Pseudomonas*. Methanol 90% extract showed maximum ZOI of 22 mm against *Escherichia coli*. While cold water and ethyl acetate extracts showed minimum effective against *Escherichia coli*, the ZOI were 8 mm and 7 mm respectively. All extracts had no activity against *Pseudomonas*. GC analysis of the extracts showed there are various biologically active compounds in 90% extracts of ethanol, methanol and ethyl acetate. Based on the results it can be concluded that the waste portions of the citrus fruits, the peels could be very good source for antimicrobial components. Further purification and separation steps are required to show the other phytochemical contents.

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