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Antimicrobial Activity of Different Aqueous Lemon Extracts

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ABSTRACT

Over three-quarter of the world's population is using herbal medicines with an increasing trend globally. Plant medicines may be beneficial but are not completely harmless. The aim of this study is to evaluate the antimicrobial activity of different types and part of lemon against different microbial isolates. The antimicrobial effects of aqueous extracts of peel and juice from fresh and dried citrus and sweet lemon against 6 Gram-positive and 8 Gram-negative bacterial and one yeast isolates, including *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Enterococcus faecalis, Streptococcus pneumoniae, Streptococcus agalactiae, Pseudomonas aeruginosa, Enterobacter aerogenes, Klebsiella pneumoniae, Escherichia coli, Salmonella Typhi, Proteus spp., Moraxella catarrhalis, Acinetobacter spp. and Candida albicans,* all of them were studied. The water extracts of all the materials screened showed various inhibitory effects. The juice of *Citrus limon* has antimicrobial activities more than other types of extracts. *Escherichia coli, Staphylococcus epidermidis, Streptococcus agalactiae and Candida albicans* showed the highest resistance to these extracts. Lemon species might have antimicrobial activity against different Gram-positive, Gram-negative and yeast pathogens and could be used for prevention of various diseases caused by these organisms.

INTRODUCTION

For a long period in history, plants have been valuable and indispensable sources of natural products for the health of human beings and they have a great potential for producing new drugs (Nascimento *et al.*, 2000). Bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Abeysinghe, 2010). Finding new naturally active components from plants or plant-based agricultural products has been of interest to many researchers. Hence, a great deal of attraction has been paid to the antibacterial activity of citrus as a potential and promising source of pharmaceutical agents (Jo *et al.*, 2004; Ortuño *et al*., 2006). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Nascimento *et al.*, 2000). Lemon is an important medicinal plant of the family Rutaceae. It is used mainly for its alkaloids, which are having anticancer activities and the antibacterial potential in crude extracts of different parts (leaves, stem, root, juice, peel and flower) of Lemon against clinically significant bacterial strains has been reported (Kawaii *et al.*, 2000). Citrus flavonoids have a broad spectrum of biological activity including antibacterial, antifungal, anti-diabetic, anticancer and antiviral activities (Burt, 2004; Ortuño *et al*., 2006). Antimicrobial activity of the peel extract is directly concerned with the components that they contain.

The studies showed that essential oils, protopine and corydaline alkaloids, lactons, polyacetylene, acyclic sesquiterpenes, hypericin and pseudohypericin compounds are effective toward various bacteria (Keles *et al.*, 2001; Maruti *et al.*, 2011). Furthermore, citrus fruit had been used in traditional Asian medicines for centuries to treat indigestion and to improve bronchial and asthmatic conditions (Kalpa *et al.*, 2012). Johann *et al*., (2007) and Ghasemi *et al.*, (2009) have shown that citrus varieties are considered and containing a rich source of secondary metabolites with the ability to produce a broad spectrum of biological activities.

Giuseppe *et al*., (2007) have reported the presence of limonoids in *Citrus* species, which can be considered responsible for activity against many clinically, isolated bacterial strains.

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Limonoids obtained from *C. limon*, showed good antibacterial and antifungal activity. Extracts of citrus fruit (e.g. lemon, orange and grape fruit) are among the most studied natural antimicrobials for food applications), and it has shown to be effectively decrease the growth of bacteria (Corbo *et al.*, 2008). There are several *Citrus (C.)* species, of these *C. limon* (lemon), *C. aurantium* (bitter orange), *C. limetta* (sweet lemon), *C. jambhiri* (Rough lemon) and *C. paradise* (grape fruit) (Al-Ani *et al.*, 2009).

Due to rapid increase of antibiotic resistance in our country, plants that have been used as medicines over hundreds of years, constitute an obvious choice for study. It is interesting to determine whether their traditional uses are supported by actual pharmacological effects or merely based on folklore. The aim of this study is to evaluate the antimicrobial activity of different types and part of lemon against different bacterial isolates.

MATERIALS AND METHODS

Microbial isolates

Different fifteen clinical microbial isolates (Gram positive, Gram negative and yeast) (listed at table-1) were isolated and identified by using conventional biochemical tests and Api system (Biomeraux, France) (Forbes *et al.*, 2007) and cultivated in pure culture, at microbiological laboratory/college of Medicine / Babylon University.

Table. 1: Bacterial isolates used in this study.

Gram positive bacterial isolates	Gram negative bacterial isolates	Fungi
***Staphylococcus* species** *Staphylococcus aureus* *Staphylococcus epidermidis* **Streptococcus species** *S. agalactiae* *Enterococcus faecalis* *Streptococcus pneumoniae* *Streptococcus pyogenes*	**Enterobacteriaceae** *Escherichia coli* *Enterobacter aerogenes* *Klebsiella pneumoniae* Proteus spp. *Salmonella Typhi* **Gram negative cocci** *Acinetobacter spp.* *Moraxella catarrhalis* *Pseudomonas aeruginosa*	*Candida albicans* (yeast)

Plants collection

Fresh and dried fruits listed at table (2) used in this study were obtained from the local market at Hilla City, Iraq, 2013. The fresh fruits were washed in running tap water in laboratory, surface sterilized with 70% alcohol, rinsed with sterile distilled water and cut open with a sterile knife and the juice pressed out into a sterile universal container separately and then filtered (using Millipore 0.45 filter paper) into another sterile container to remove the seeds and other tissues and used freshly as crude without refrigeration. Extracts of peels were stored at 4 °C until use.

Table. 2: Medicinal plants used to evaluate antibacterial activity.

s. no	Scientific name	English name	Parts of plant used	Local Arabic name
1.	*Citrus limon L.*	Lemon	Fruit (peel)	نومي حامض
2.	*Citrus limon L.*	Lemon	Fruit (juice)	
3.	*Citrus limon L.*	Lemon	Fruit(driedfruit)	نومي بصرة
4.	*Citrus limetta*	Sweet lemon	Fruit (peel)	نومي حلو
5.	*Citrus limetta*	Sweet lemon	Fruit (juice)	

Antimicrobial activities

The screening of antimicrobial activities of each crude aqueous lemon extract on the tested bacteria used in this investigation was determined on Muller Hinton agar media (all tested organism grow on Muller Hinton agar media), by the using agar well diffusion method. Wells of 6 mm diameter and 5 mm depth were made on the solid agar using a sterile glass borer (CLSI, 2002; Prescott *et al.*, 2002).

Approximately 20µl of each extract was inoculated onto wells were made in the spread plate culture of each microbial isolates. (The plates were performed in triplicates). All plate of the tested organisms was then allowed to incubate at 37°C for overnight. After 24 h of incubation, each extract was noted for zone of inhibition for all isolates. The diameters of the zone of inhibitions were measured by measuring scale in millimeter (mm).

Statistical analysis

Bonferroni test recommended by Danial (1988) was used for statistical analysis ($P \leq 0.05$) to show if there is any significant differences between lemon extracts.

RESULTS AND DISCUSSION

Results of antimicrobial activity of lemon extracts against Gram positive and negative isolates by the agar well diffusion method were shown on Table (3) and (4) respectively. The microbial susceptibility was collectively summarized in Figure (1). The water extracts of all the materials screened showed various inhibitory effects.

Table. 3: Antimicrobial activity of lemon extracts against Gram positive and yeast isolates measured in (mm).

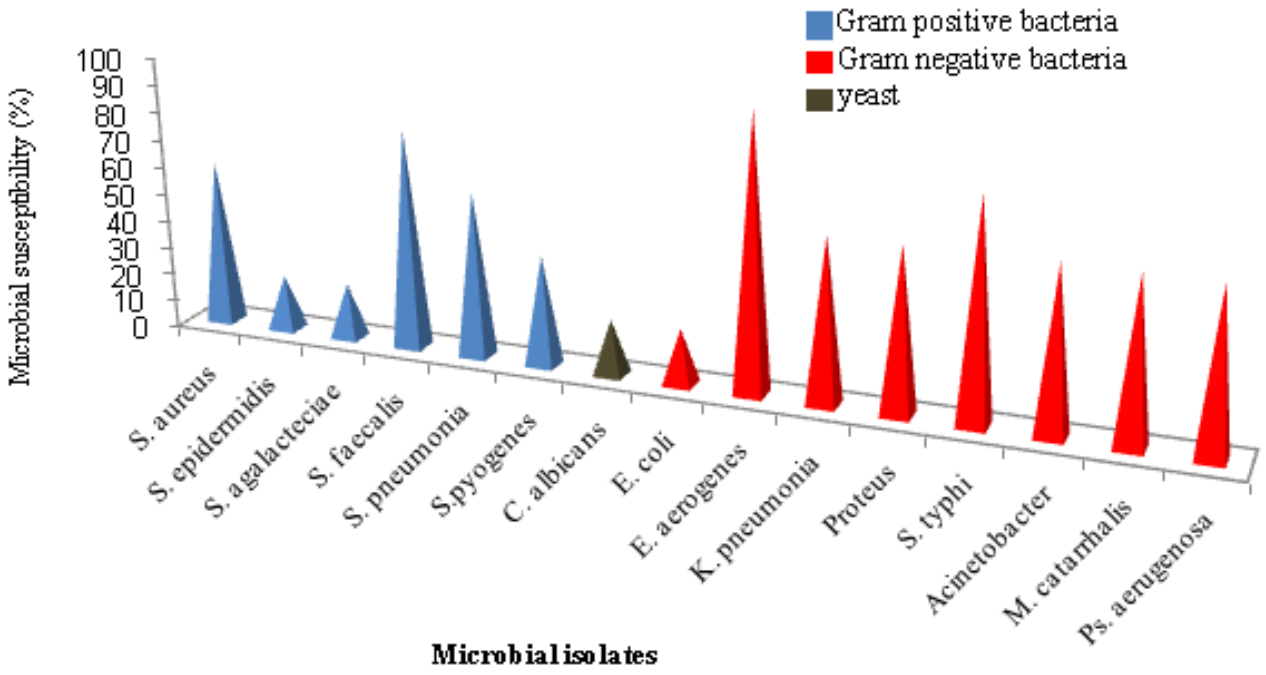
microbial isolates	Lemon extracts				
	***Citrus limon* (peel)**	***Citrus limon* (juice)**	***Citrus limon* (dry)**	***Citrus limetta* (peel)**	***Citrus limetta* (juice)**
S. aureus	30	26	N.I	20	N.I
S. epidermidis	N.I	N.I	25	N.I	N.I
S. agalactiae	N.I	20	N.I	N.I	N.I
Enterococcus faecalis	30	28	28	25	N.I
S. pneumoniae	N.I	29	18	35	N.I
S. pyogenes	N.I	20	N.I	24	N.I
Candida albicans	N.I	30	N.I	N.I	N.I

mm= millimeter (diameter of inhibition zone), N.I= No Inhibition

Table. 4: Antimicrobial activity of lemon extracts against Gram-negative isolates measured in (mm).

Bacterial isolates	Lemon extracts				
	***Citrus limon* (peel)**	***Citrus limon* (juice)**	***Citrus limon* (dry)**	***Citrus limetta* (peel)**	***Citrus limetta* (juice)**
Escherichia coli	N.I	10	N.I	N.I	N.I
E. aerogenes	20	20	20	20	30
K. pneumoniae	20	30	20	N.I	N.I
Proteus spp.	N.I	20	20	N.I	20
S. Typhi	N.I	30	8	20	20
Acinetobacter spp.	N.I	20	N.I	10	10
M. catarrhalis	30	30	N.I	20	N.I
Ps. aeruginosa	N.I	N.I	10	10	10

mm= millimeter (diameter of inhibition zone), N.I= No Inhibition



The extract from the juice of *C. limon* presented the highest antimicrobial activities, as it inhibited most 13 isolates (out of 15 isolates used) of the bacteria under the study whether Gram positive or negative with inhibition zone ranging from (10-30mm) except *S. epidermidis* and *P. aeruginosa* which showed no any susceptibility to this extract. On the other hand, the extracts from the peel of *C. limon* show low antimicrobial activity inhibited only 5/15 isolates (out of 15 isolates used), while the juice of *C. limetta* showed no any activity against Gram-positive bacteria but inhibited 5 Gram-negative bacteria (out of 8 isolates used).

Drastically, peels of *C. limetta* produced a good antimicrobial activity (Out of 15 isolates used) with inhibition zones rang (10-35mm) whether against Gram-positive or Gram-negative bacteria with no response against *Candida*.

E. aerogenes showed the highest susceptibility (out of Gram-negative bacteria) to all of the extracts, with inhibition zones (20-30mm). Followed by *S. Typhi* was susceptibility to 4 extracts (out of 5 extracts used). Followed by *K. pneumoniae*, *Proteus*, *Acinetobacter* and *M. catarrhalis* were resistance to 3 extracts (out of 5 extracts used).

Among Gram-positive isolates, *Enterococcus faecalis* gave the top susceptibility to most of the 4 extracts (out of 5 extracts used). Followed by *S. aureus* and *S. pneumoniae* was susceptibility to 3 extracts (out of 5 extracts used). Last but not least *S. pyogenes* was susceptibility to 2 extracts (out of 5 extracts used).

Statistical analysis showed significant differences between effect of *C. limon* (peel) and *C. limon* (juice), extract on microbial isolates, there were no significant differences between *C. limon* (peel) and *C. limon* (dry) extract on microbial isolates, and there were no significant differences between *C. limon* (peel), and *C. limetta* (peel) on microbial isolates, and there were no significant differences between *C. limon* (peel) and *C. limetta* (juice) at level ($P \leq 0.05$). Statistical analysis showed significant differences between effect of *C. limon* (juice), and *C. limon* (peel), extract on microbial isolates, there were significant differences between *C. limon* (juice) and *C. limon* (dry) extract on microbial isolates, and there were significant differences between *C. limon* (juice) and *C. limetta* (peel) on microbial isolates, and there were significant differences between *C. limon* (juice) and *C. limetta* (juice) at level ($P \leq 0.05$).

Statistical analysis showed significant differences between effect of *C. limon* (dry), and *C. limon* (juice), extract on microbial isolates, there were no significant differences between *C. limon* (peel) and *C. limon* (dry) extract on microbial isolates, and there were no significant differences between *C. limon* (dry), and *C. limetta* (peel) on microbial isolates, and there were no significant differences between *C. limon* (dry) and *C. limetta* (juice) at level ($P \leq 0.05$).

Statistical analysis showed no significant differences between effect of *C. limetta* (peel), and *C. limon* (juice) extract on microbial isolates, there were significant differences between *C. limetta* (peel), and *C. limon* (dry) extract on microbial isolates, and there were no significant differences between *C. limetta* (peel), and *C. limon* (peel) on microbial isolates, and there were no significant differences between *C. limetta* (peel), and *C. limetta* (juice) at level ($P \leq 0.05$). Statistical analysis showed on significant differences between effect of *C. limetta* (juice) and *C. limon* (juice), extract on microbial isolates, there were significant differences between *C. limetta* (juice) and *C. limon* (dry) extract on microbial isolates, and there were no significant differences between *C. limetta* (juice) and *C. limetta* (peel) on microbial isolates, and there were no significant differences between *C. limetta* (juice) and *C. limon* (peel), at level ($P \leq 0.05$).

The microorganism *E.coli*, which is already known to be multi-resistant to drugs, was also resistant to the plant extracts tested. It was susceptible only to the juice of *C. limon*. Similar result was noted with *S. epidermidis* with vulnerability only to the extract of dry *C. limon*, *S. agalactiae* and *C. albicans* to the juice of *C. limon*.

On the other hand, *P. aeruginosa*, which is also resistant to different antibiotics, its growth was inhibited weakly by the extracts from peel and juice of *C. limetta* and dry lemon.

The prevalence of antibiotic resistance is a continual problem due to the evolution of a potent defense mechanism against antibiotics. Therefore, it is necessary to exploit and develop a novel inhibitory agent against those bacteria (Cabello, 2006). Plants and plant products have been used extensively throughout history to treat medical problems. Numerous studies have been carried out to extract various natural products for screening antimicrobial activity (Nita *et al*., 2002).

The results indicated that the extracts of all the sorts studied showed antibacterial activities towards the Gram-positive, negative bacteria and yeast, but with variability related to the bacterial genus and species.

Some significant components are abundantly available in citrus peel, including ascorbic acid, phenolic acids, polyphenols, and dietary fiber (Gorinstein *et al.,* 2001). Constituents with antioxidant, antiviral, antibacterial, antifungal, and anticancer activities have also been reported in citrus (Matasyoh *et al.,* 2007; Mahmud *et al.,* 2009). Numerous studies have described the inhibitory activities of citrus against human pathogens, fungi, and yeasts and food pathogens (Nannapaneni *et al.,* 2008, Lee and Najiah, 2009).

The reason for the different sensitivity of the Gram-negative bacteria compared to that of Gram-positive bacteria could be due to differences in their cell wall composition. Gram-positive bacteria contain an outer peptidoglycan layer, which is an effective permeability barrier, whereas Gram-negative bacteria have an outer phospholipidic membrane (Samarakoon *et al.*, 2012).

Hayes and Markovic (2002) investigated the antimicrobial properties of lemon and found that lemon possesses significant antimicrobial activity against *S. aureus*, *Klebsiella*, *Escherichia coli, P. aeruginosa* and *C. albicans*. Nevertheless, these results unmatched with our results as these organisms showed resistance to most of these extracts except that of *S. aureus* and *Klebsiella* as it matched with these results with inhibition zone (20-30mm). Moreover, Al-Ani *et al* (2009) showed good bacterial inhibition by *C. limon* especially against *S. aureus, P. aeruginosa* and *P. vulgaris*.

In addition, Abdullah (2009) found that the juice of C. *limon* has significant inhibition against both *S. aureus* and *K. pneumoniae* with inhibition zones 17.4 and13.3 mm respectively, While the juice of C. *limetta* gave no any inhibition effect on these bacteria. These results were agreed with our results as the juice of C. *limon* was more effective. This could be due to the acidic pH of this juice that will affect the charges of the amino acids that constitute the peptidoglycan, and it may affect the active sites of enzymes leading to defect in their activity (Abdullah, 2009).

The resistance of Gram-negative bacteria to plant extracts was not unexpected. In general, this class of bacteria is more resistant than Gram-positive bacteria. Such resistance could be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism (Abu-shanab *et ai.*, 2004). Infections caused by *P. aeruginosa*, especially those with multi-drug resistance, are among the most difficult to treat with conventional antibiotics (CDC, 1999). In this study, the growth of *P. aeruginosa* was slightly inhibited by lemon extracts. Such results are very interesting and with expectation, increasing the concentration of the extracts may produce more inhibition to this bacterium. They may inhibit bacteria by a different mechanism than that of currently used antibiotics and may have therapeutic value as an antibacterial agent against multi-drug resistant bacterial strains.

There are clinical studies found that daily usage of pure hand gel which contain *C. limon* in its ingredients is associated with reduction in the microbial load, which is important to prevent risk of transmitting nosocomial infections by healthcare workers (Kavathekar *et al.*, 2004; Ravikumar *et al.*, 2005). This result agreed with our outcome as it showed that *S. Typhi murium* is affected with all these extracts except the peel of *C. limon*.

Dhanavade *et al*., (Dhanavade *et al.*, 2011) suggest that different alcoholic extracts of lemon peel give antimicrobial activity against different bacterial isolates especially *P. aeruginosa* and *S. Typhi murium* better than the aqueous extract that we used in this study as it gave no effect against most of the study isolates including the two mentioned above.

Generally, Rahman *et al* (2011) documented that, during the detection of microbial susceptibility to different plant extracts, the size of inhibition zone to indicate relative antibacterial activity is not adequate. The zone must be affected by the solubility and rate of diffusion in agar medium or its volatilization; and thus the results could be affected.

CONCLUSION

Lemon extracts have an important role as antimicrobial agents against microorganisms. They are natural, cheap, safe, and due to increase antibiotic resistance among bacteria.

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