Plant Archives Vol. 18 No. 1, 2018 pp. 431-434 *ISSN 0972-5210*

AQUEOUS LEMON EXTRACTS AS ANTI MICROBIAL AGENT AGAINST SOME PATHOGENIC BACTERIA

Iman Fadhil Abdul-Husin[1], Sharafaldin Al-musawi[1], A. Nada Khazal Kadhim Hindi[2] and Salih Abudl-Mahdi[1]

[1]College of Biotechnology, Al-Qasim Green University, Babylon, Iraq.
[2]Department of Basic and Medical Science, College of Nursing, University of Babylon, Babylon Province, Iraq.
E-mail : fadhel_iman @yahoo.com, Dr.sharaf@biotech.uoqasim.edu.iq , nadakhazal@yahoo.com, foratyoon@yahoo.com

Abstract

Herbal medicines are plants have been used by over three-quarter of the world's population due to the fact of their possibility to be beneficial but are not completely harmless. the aim of this study is to evaluate the antimicrobial activity of lemon aqueous extract against different bacterial isolates. The antimicrobial effects was studies against 5 Gram-positive and5 Gram-negative bacteria, including; *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhi*. The water extracts of all the materials screened showed various inhibitory effects. *Escherichia coli*, *Staphylococcus epidermidis*, showed the highest resistance to both extracts. Antimicrobial activity of Lemon extract against different Gram-positive, Gram-negative pathogens could be used for prevention of various diseases caused by these organisms.

Key words : Antimicrobial, lemon, aqueous extract, *Citrus limon* L., *Citrus limetta*.

Introduction

Acquire resistance of bacteria to drugs which are utilized as therapeutic agents due to bacterial genetic ability to change (Abeysinghe, 2010) made the finding of new components that are naturally active from plants or plant-based agricultural products as a matter of interest to many researchers. Lemon is an important medicinal plant of the family Rutaceae which mainly well known for its alkaloids that have been used medically as anticancer activities and the potential antibacterial activity against significant bacterial strains (Kawaii *et al*., 2000). Abroad spectrum of biological activity Citrus flavonoids including antimicrobial, anticancer as well as anti-diabetic activities have been reported by Burt (2004) and Ortuño *et al*. (2006). Other studies reported by Keles *et al*. (2001) and Maruti *et al*. (2011) reveals that protopine and corydalinelactons, polyacetylene, acyclic sesquiterpenes, hypericin pseudohypericin and essential oils o compounds of (*Citrus lemon* L.) Peel Extract are effective toward various bacteria while the presence of limonoids in *Citrus* species have been consider the responsible for antimicrobial activity against many clinically, isolated bacterial strains reported by Giuseppe *et al*. (2007). Citrus family have a medicinal and cooking importance and its largely varied in Iraq i.e *Citrus limon* L., *Citrus limetta*, *Citrus aurantium*, *Citrus sinensis*, *Citrus reticulata* and *Citrus grandis*. In this search, *Citrus limon* L., *Citrus limetta* antibacterial activity has been checked in terms of MIC against most Popular pathogenic isolated bacteria in Babylon provence.

Materials and Methods

Bacterial isolates

Different ten clinical bacterial isolates (Gram positive, Gram negative) were isolated from clinical samples of patient whom Fallen asleep in Al-Hashimia hospital, Babylon during 2016and identified by using conventional biochemical tests and Api system (Biomeraux, France) and cultivated in pure culture at microbiological laboratory/ college of Biotechnology, Al-Qasim Green University.

Plant samples

Fresh fruits of *Citrus limon* L., *Citrus limetta* were obtained from the local market at Hilla City, Iraq, 2016. After washed in running tap water in laboratory; then the surface of fruit was sterilized with 70% alcohol, which rinsed with sterile distilled water. By sterile knife; the fruit were cutand the juice pressed out into a sterile universal container separately; then filtered using Millipore 0.45 filter paper in another sterile container to remove the seeds and other tissues. The freshly crude obtained without refrigeration and stored at 4^0C for subsequentuse.

Antimicrobial activities test

Agar well diffusion method was the method that use in this work, in which Mueller-Hinton agar was used to cultured tested bacterial isolates due to its better diffusion of the extractswhichleads to a truer zone of inhibition since it's a loose agar as well as it is a non-selective, non-differential medium. After incubation, a sterile cork borer was used to cut the agar surface and three wells with 7 mm diameter were made .20 µl of the plant extracts were added to each plate wells. The incubation was at 37°C for 24 hours. Confluent bacterial growth was observedand the antibacterial activity was assayed by calculate the inhibition zone diameter that formed around the wells (Prescott *et al*., 2002).

Determination of minimum inhibitory concentration

The MIC value was measured according by Owoseni and Ajayi (2010) in which different concentrations of two Lemon species aqueous extract (serial dilutions of the extracts was prepare (100, 50, 25 and 12.5%). The tubes were then incubated for 24 h at 37°C. Lowest concentration of the extracts that did not exhibit any visible growthwas determined as MIC value of extracts against tested bacteria.

Minimum bactericidal concentration

The MBC value of the water extracts was determined according to CLSI (2008). No turbidity tubes in MIC test were cultured (a loop full amount) again on Mueller–Hinton agar and incubated for 24 h at 37°C. The concentration of the extracts with no growth was regarded as the minimum bactericidal concentration (MBC).

Statistical analysis

The data were subjected to one way analysis of variance and Statistical analysis was applicable according to (SPSS Statistics analysis systems 17.0 Inc.). $P \leq 0.05$ were regarded as significantto show if there are any significant differences between lemon extracts.

Results and Discussion

Antimicrobial activity measuring by inhibition zone diameter of the two Lemon species aqueous extract against Gram positive and negative against tested bacteria were shown in table 1.

From table 1; result of the present study illustrate that *Citrus limon* L have more activity than *Citrus limetta* since it was active against 7 out of 10 of tested bacteria compare to *Citrus limetta*, which were active against only 5 isolates of tested bacteria with no significant differences on microbial isolatesat level ($P \leq 0.05$). *Escherichia coli* and *Staphylococcus epidermidis* showed the highest resistance to both extracts which well known to be multi-resistant to drugs bacteria. On the other hand, *P. aeruginosa*, *Enterobacter aerogenes*, which is also resistant to different antibiotics (CDC NNIS System, 1999), its growth was inhibited by both extracts. Significant inhibition of *Staphylococcus aureus* and *K. pneumonia* bacteria were obtained in this study in both limon extract and were accordance with Abdullah (2009), which may be related to the high (acidic) pH of this juice that will effect on the charges of the amino acids that constituent the peptidoglycan layer of bacterial cell or may be due to the effect on active sites of enzymes leading to defect in their activity. In this study; different sensitivity of the Gram-ve bacteria and Gram+ve bacteria because of their variances in cell wall composition (Samarakoon *et al*., 2012). *C. limon* in ingredients is associated with reduction in the microbial load, which is important to stop risk nosocomial infections to of transmitting by healthcare workers (Kavathekar *et al*., 2004) and this is agree with result of *S. Typhi*, which is affected with all these extracts. The values of MIC and MBC against tested bacteria are shown in table 2.

MIC values ranged from 12.25 to 100 µg/mL, while MBC values ranged from 50 to 200 µg/mL for all the juices studied. Obtained results showed that the MIC values for the *Citrus limon* L. and *Citrus limetta* juice concentrates were less than their MBC values. Thus, the two extracts suggesting being bacteriostatic at low concentrations and bacteriocidal at higher concentrations.

One of the most common bacteria implicated in food poisoning is *Staphylococcus aureus*. The lemon juice concentrates showed good inhibitory and bacteriocidal activities against this pathogen. *P. aeruginosa* and *Enterobacter aerogenes* and *Salmonella typhi* bacteria tested in this study significantly inhibited by lemon juice concentrates (MIC 25 µg/mL). Thus, juice concentrates may therefore hold promise in the management of infection caused by these bacteria. The highest efficacy of citrus

Table 1 : Antimicrobial activity of lemon extracts against Gram positive and negative bacterial isolates measured in (mm).

Tested bacteria	Inhibition zone (mm) of *Citrus limon* L	Inhibition zone (mm) of *Citrus limetta*
Staphylococcus aureus	21	9
Staphylococcus epidermidis	-	-
Streptococcus pyogenes	20	-
Streptococcus pneumoniae	14	-
Streptococcus agalactiae	27	11
Pseudomonas aeruginosa	20	10
Enterobacter aerogenes	20	30
Klebsiella pneumoniae	30	-
Escherichia coli	-	-
Salmonella typhi	30	20

Table 2 : MIC of tested extract against tested bacteria.

Tested bacteria	MIC value of *Citrus limon* L	MIC value of *Citrus limetta*
Staphylococcus aureus	12.5	25
Streptococcus pyogenes	25	-
Streptococcus pneumoniae	50	-
Streptococcus agalactiae	12.5	50
Pseudomonas aeruginosa	25	100
Enterobacteraerogenes	50	12.5
Klebsiellapneumoniae	12.5	-
Salmonella typhi	12.5	12.5

Table 3 : MBC of tested extract against tested bacteria.

Tested bacteria	MBC value of *Citrus limon* L	MBC value of *Citrus limetta*
Staphylococcus aureus	50	100
Streptococcus pyogenes	50	-
Streptococcus pneumoniae	100	-
Streptococcus agalactiae	25	100
Gram Negative bacteria		
Pseudomonas aeruginosa	25	100
Enterobacteraerogenes,	25	25
Klebsiellapneumoniae	25	-
Salmonella typhi	25	50

juice to inhibit bacterial cell growth in this study were concurrent with Mathur *et al.* (2011), who showed that the aqueous extracts from citrus fruit pulp had antimicrobial activity with significantly ratio. The microbicidal acids of the citrus juices are substances active-membrane which destroyed inner membrane in their unrelated form. They change the permeability of the microbial cell membrane and acidify the cytoplasm (Puupponen-Pimia *et al*., 2004).

Conclusion

Antimicrobial activity of Lemon extract against different Gram-positive, Gram-negative pathogens could be used for prevention of various diseases caused by these organisms.

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