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Antimicrobial Activity Evaluation of Citrus Lemon Against *Streptococcus Pyogenes* And *Escherichia Coli*

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Abstract:
Background: The therapeutic activities of citrus lemon have been reported in several studies including antimicrobial ,anti-inflammatory, antiparasitic and anticancer activities.
Objectives: To determine the potential antimicrobial activity of ethanol and methanol extracts of citrus lemon peel and lemon juice in inhibiting bacterial isolates**.**
Methods and Materials: The antimicrobial effects of citrus lemon alcoholic extracts of peel and juice against Gram-positive (*Streptococcus pyogenes*) and Gram-negative (*Escherichia coli*) bacterial isolates were studied**.**
Results : Citrus limon juice has antimicrobial activities more than alcoholic extracts on both bacterial isolates with *Escherichia coli* 15mm and *Streptococcus pyogenes* 20mm. The ethanol and methanol extracts of citrus limon peel showed 5 mm and 6 mm of the average inhibition zone against *Escherichia coli* respectively. On the other hand the study showed no inhibition effect of both alcoholic extracts on *Streptococcus pyogenes*.
Conclusion: This study demonstrated that citrus lemon juice can be used as a potential source for controlling *Streptococcus pyogenes* and *Escherichia coli* growth.
Keywords: Citrus lemon; lemon juice; *Escherichia coli*; *Streptococcus pyogenes*; Antimicrobial

I. INTRODUCTOION

Spreading of serious infectious pathogens is causing severe morbidity and mortality rate worldwide including *Escherichia coli* and *Streptococcus pyogenes.* As well as an alarming increase in antibiotic resistance rate is concerned [1], [2] .*Escherichia coli (E.coli)* are part of the normal bacterial flora found in the gastrointestinal tract. However, some strains of *E.coli* are capable of producing toxins that may lead to serious infections. *E. coli* bacteria strains involved in diarrheal diseases and is considered as the second leading cause of diarrhea after rotavirus [3], [4] In addition, *E.coli* can cause the majority of urinary tract infections, and it is also capable of infecting the bloodstream and meninges [5] Many studies have been reporting that multidrug resistance (MDR) in *Escherichia coli* is increasingly observed [6], [7].

Streptococcus pyogenes (S. pyogenes) known as (group A streptococcus) is responsible for a various range of clinical presentations from less serious diseases including sore throat, pharyngitis, tonsillitis, scarlet fever, impetigo and cellulitis, to more serious diseases, such as sepsis, meningitis, pneumonia and necrotizing fasciitis [8], [9]. Although there is a decreasing in the incidence of *S. pyogenes* diseases in developed countries as a result of improved living conditions, many studies reveled that *S. pyogenes* strains are resistant to certain antibiotics such as erythromycin, clindamycin, tetracycline and fluoroquinolone [10], [11].

Thus, the previous issues prompt the scientists switch to new approach of treatment. Plants, over the history, acquiring biological ingredients showed a beneficial impact on human being health with least toxicity. These natural substances have a considerable possibility in producing new drugs [12]. Citrus plants extracts contain metabolites which are mainly phenolic derivatives, these derivatives inhibit bacterial growth by binding to bacterial protein or reducing pH without any resistance reported [13].

Many studies reported the therapeutic activities of citrus lemon (limon) including anti-inflammatory, antimicrobial, anticancer and antiparasitic activities due to the presence of a high content of phenolic

compounds, mainly flavonoids (e.g., diosmin, hesperidin, limocitrin) and phenolic acids (e.g., ferulic, synapic, p-hydroxybenzoic acids) [5], [13], [14]. Additionally, the peels of citrus limon are used in therapeutic agents of different diseases such as scurvy, respiratory problems, indigestion ,eye infection, skin diseases since it has a full different types of nutrients and phyto-chemicals which include β- and γ-sitosterol, different types of volatile oils and glycosides[15], [16]. The present study was aimed to determine the antibacterial activity of ethanol and methanol extracts of citrus lemon peel and lemon juice, against standard bacterial cultures *S. pyogenes* and *E. coli*.

II. MATERIALS AND METHODS

This study conducted in the microbiological laboratory, department of biomedical science, faculty of pharmacy , Omar Al-Mukhtar university.

2.1 Preparation of citrus lemon peel extracts

Citrus lemon fruit was collected from the local garden of El-Beida, northeastern Libya, in April, 2021. Method described by Shakya et al [17] was followed with slight modifications The lemon peels were oven dried at 55°C, crushed into coarse powder with the help of blender and stored at room temperature for future use. The yield peels powder were extracted by ethanol 70% and methanol 70% (5gm powder mixed with 100ml ethanol and 5gm powder mixed with 100ml methanol for) and the mixture was kept at 30ºC for 72 hours with constant stirring. Then the extracts filtered through Whatman No.1 filter papers, the filtrate was then assessed for their antimicrobial activity

2.2 Preparation of citrus lemon juice

The lemon fruit were washed by running tap water in laboratory, outer surface is sterilized by 70% alcohol, rinsed by sterile distilled water then cut by sterile knife and the juice pressed out into a sterile universal container separately. The obtained juice is filtered using filter paper (Millipore 0.45) into another sterile container to remove the seeds and other tissues and use immediately.

2.3 Microbial isolates and antibacterial activities of citrus lemon

Two different clinical bacterial isolates Gram positive *S. pyogenes* and Gram negative bacteria *E.coli*, were isolated and identified by using conventional biochemical tests and Api system (Biomeraux, France) [18] at Al Burj medical laboratory, El-Beida, Libya. The antimicrobial activities of the different extracts of the citrus lemon was performed by disc diffusion assay. The two bacterial isolates were transferred into 13 ml sterile nutrient broth and incubated at 37ºC for 18-24 hours. A 0,1 ml of the broth were inoculated into the Petri dish and add in 15 ml nutrient agar and were mixed until the nutrient agar solidified. The blank discs (diameter 5 mm) were dipped into the 10µl of each extract stock solution and then plated on the surface of nutrient agar of each Petri dish. Ciprofloxacin and Gentamicin antibiotic discs were used as a control. After 24 hours of incubation, the inhibition zones were determined using measuring scale in millimeter (mm). [19]

III. RESULTS

The sensitivity of , *E.coli* and *S. pyogenes* against the ethanol and methanol extracts of citrus lemon peel and lemon juice samples studied was determined. **Table 1** shows the diameter values of inhibition of isolated microorganisms.

Table 1 Antimicrobial activity of citrus lemon alcoholic extracts and Lemon juice against *E.coli* and *S. pyogenes*

Citrus lemon extracts and juice	Zone of inhibition (in mm)	
	E. coli	*S. pyogenes*
Ethanol extract	5	0
Methanol extract	6	0
Lemon juice	15	20

The results of this study were also compared with the inhibition zone diameters of selected antibiotics Ciprofloxacin and Gentamicin **Table 2**

Table 2 Antibacterial activity of Ciprofloxacin and Gentamicin against *E. coli* and *S. pyogenes*

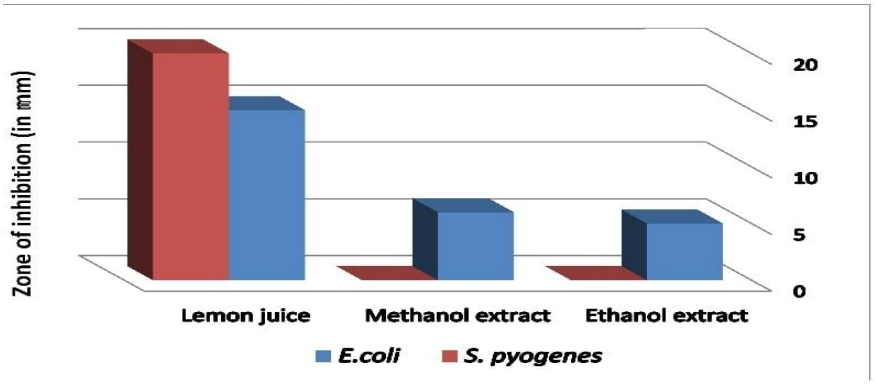
Antibiotic types	Zone of inhibition (in mm)	
	E .coli	*S. pyogenes*
Ciprofloxacin	0	34
Gentamicin	14	19

IV. DISCUSSION

The antimicrobial, antioxidant and anticancer activities of citrus lemon have been confirmed in several studies [5], [13], [14], [20], the compounds responsible for the antibacterial action of citrus lemon are flavonoids, carotenoids, limonoid, tannin, and terpenoids.[21], [22].

The present study attempted to evaluate the antibacterial activity of citrus lemon as a medicinal plant and to what extent could be used as a therapeutic agent for infectious diseases to overcome antibiotic resistance dilemma. As a result for *Escherichia coli* isolate, the antimicrobial activity of ethanol and methanol extract of the citrus limon peel (70%) showed 5 mm and 6 mm of the average inhibition zone against *E. coli* respectively.

Figure 1 Antibacterial activity of Ciprofloxacin and Gentamicin against *E. coli* and *S. pyogenes*



However, several earlier studies reported higher antimicrobial activity of the ethanol extract of the citrus limon peel with different concentrations against *E. coli* ranged from 15 mm to 18.77 mm of inhibition zone average [5], [19], while the inhibition zone was 20 mm of ethanolic extract on *E. coli* [23]. Additionally, Abdel–Salam and Mostafa 2014 stated that aqueous lemon extract also had antibacterial effect with 11 mm [24]. Further study performed by Al-Snafi,2019 stated that aqueous and ethanolic extract of citrus limon have antimicrobial activity against *E. coli* with diameter of inhibition zone of 11 and 18 respectively [25]. On the other hand, another study showed no inhibition effect of citrus limon peel extract of Aqueous on *E. coli* [26]

Regarding *S. pyogenes*, antimicrobial activity of ethanol and methanol extract of the citrus limon peel of 70% showed 0 mm of average inhibition zone for both extract in the present study. Hindi and Chabuck, 2013 reported similar result as *S. pyogenes* showed no inhibition zone by aqueous extract of the citrus limon peel [26]. One more study conducted by Mathur *et al.*,2011 concluded that citrus maxima (chakotara) had antimicrobial activity against *S. pyogenes* with water and ethanolic extracts with13 mm and 8 mm inhibition zone respectively [27]. Inadequate studies have used methanol extract of the citrus limon peel compared to ethanol, however methanol extract showed promising antibacterial activity against pathogenic bacteria such as *Staphylococcus aureus* and Bacillus cereus [28]

The result for citrus lemon juice showed the highest antimicrobial activities against both bacterial isolates, with *E. coli* 10 mm and *S. pyogenes* 20mm, similar result was noted by Hindi and Chabuck, 2013 as citrus lemon juice inhibited 13 isolates (out of 15 isolates used in the study) of the bacteria whether Gram positive or negative with inhibition zone ranging from 10-30mm, including *E. coli* 10 mm and *S. pyogenes* 20 mm [26]. Mathur *et al.*,2011 stated that antimicrobial activity of pulp extract of citrus maxima (chakotara) against *S. pyogenes* with water, ethanolic and chloroform extracts were 14 mm, 11 mm and 10 mm respectively[27].

The antibiotics selected (Ciprofloxacin and Gentamicin) in this study were used as a control since they are the choice of treatment for infectious diseases caused by tested bacteria (*E. coli* and *S. pyogenes*). respectively. Gentamicin has been widely used in medicinal applications to treat *Streptococcus* infectious disease with primary and secondary skin infection [29]. Ciprofloxacin is a broad spectrum antibacterial drug that is effective against many Gram-negative and Gram-positive bacteria including *E. coli* involved in the majority

of urinary tract infections and diarrheal diseases [5], [30].

Al-Snafi, 2019 reported that, the standard antibiotics such as ciprofloxacin , gentamicin, ofloxacin and tobramycin were screened under similar conditions with citrus species to compare their activity against Gram-positive and Gram-negative bacteria[25]. In the present study antibacterial activities of Gentamicin and Ciprofloxacin were showed an inhibition zone of 19 mm and 34 mm respectively for S. pyogenes isolate. In case of *E. coli* showed 14 mm of inhibition zone with Gentamicin, while no inhibition zone against ciprofloxacin reported. A study performed by Unnisa *et al*, 2012 had moderately similar result against *E. coli* with 9 mm for Gentamicin whereas 10 mm for Ciprofloxacin [23].

Fasugba, *et al.* 2015 reported that many studies revealed the resistance pattern of *E. coli* against ciprofloxacin recently [31] That was quite different to the antimicrobial activity of lemon extracts and juice against the same isolate of *E. coli* indicating the potential use of lemon ingredients to treat infectious disease coping antibacterial resistance issue.

V. LIMITATIONS

Further studies can performed using different parts of lemon with several solvents at different concentrations such as aqueous , Petroleum ether ,acetone and ethyl acetate extract of the Citrus limon to assess its antibacterial activities.

Using Mueller Hinton agar (MHA) is preferred for antimicrobial susceptibility testing compared to nutrient agar(NA), as the use of nutrient agar showed some errors and discrepancy in the results obtained [32]

VI. CONCLUSION

The present study highlighted that using citrus lemon juice and alcoholic extract of lemon citrus peel extract can be a potential source for controlling *S. pyogenes* and *E. col*i growth. The alcoholic extract of lemon citrus peel extract was less effective than its respective citrus lemon juice against *E. coli* with no observed activity against *S. pyogenes.* As a further studies, antimicrobial effects of the citrus lemon extracts against different microbial isolates such as Staphylococcus sp, Pseudomonas sp, Klebsiella sp and Salmonella can be evaluated..

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CONFLICTS OF INTREST

The author declares that there are no conflicts of interest

Abbreviations

E. coli: *Escherichia Coli*; MHA: Mueller Hinton agar; NA: nutrient agar ; *S. Pyogenes*: *Streptococcus Pyogenes*;

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