EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 7
17-21
Year: 2017

ANTIMICROBIAL EFFECT OF BANANA PEEL AND LEMON FRUIT JUICE EXTRACTS

Rana A. Mohsien*

Department of Molecular and Medical Biotechnology, College of Biotechnology, Al-Nahrain University, Iraq.

***Corresponding Author: Dr. Rana A. Mohsien**
Department of Molecular and Medical Biotechnology, College of Biotechnology, Al-Nahrain University, Iraq.

Article Received on 25/04/2017 **Article Revised on 16/05/2017** **Article Accepted on 07/06/2017**

ABSTRACT
Several of medicinal plants are screened for potential antimicrobial activity such as banana (*Musa paradisiaca*) and lemon (*Citrus limonum*) because of the increasing failure of chemotherapeutics and the antibiotic resistance exhibited by pathogenic microbial infections. This study aimed to evaluate the antibacterial activity of alcoholic banana peel and lemon juice extracts against Gram negative (*Klebsiella pneumoniae*, *pseudomonas aeroginosa*) and Gram positive (*Staphylococcus aureus*, *streptococcus mutans*) bacteria isolated from human infections, the procedure was carried out by well agar diffusion method. The fresh extract of banana peel showed good inhibitory effect only on (*pseudomonas aeroginosa*) with no effect against other types of organism while lemon juice extract showed good inhibitory effect on all tested bacteria, but the mixture (synergistic effect) between banana and lemon extracts show a significant inhibitory effect against all tested bacteria. These results can be useful for future prospects to replacement of medicine in treatment of infections caused by these pathogenic bacteria.

KEYWORDS: *Musa paradisiaca*, *Citrus limonum*, Antimicrobial Effect, *Klebsiella pneumoniae*, *pseudomonas aeroginosa*, *Staphylococcus aureus*, and *streptococcus mutans*

INTRODUCTION

Medicinal plants were used for many years ago, to treat infectious diseases for replacement of antibiotics. In recent years there has been good attention for detection of new antimicrobial compounds; because of highly rate of infections with multi-drug resistant microorganisms especially MRSA (Methicillin resistance *Staphylococcus aureus*) (Bassam et al. 2006; Bhavani and Ballow, 2000). The reason for widely use of plants as drugs may be the fact that plants are available everywhere and thus are used without any trouble or side effects. All medicinal plants produce important secondary metabolites like terpenoids, flavonoids, polyphenols and phenolic compounds that are considered to be the chief plant constituent because of its important role as antioxidant, anti-inflammatory properties and antimicrobial agents (Nicholson and Hammerschmidt, 1992). Banana is a tropical fruit grown in different countries in all over the world. In recent times, peel of banana has been used for various industrial applications including cosmetics, bio-fuel production, pulp and paper, organic fertilizer, energy related activities, biotechnology related processes and environmental cleanup (Morton, 1987; Gunaseelan, 2004; Bori *et al*., 2007, Husain and William, 2010). All parts of the banana plant have medicinal applications; the flowers in bronchitis and dysentery and on ulcers; cooked flowers are given to diabetics (Amit and Shailandra, 2006). Many studies had been found that peel and pulp of bananas are rich sources of antimicrobial and antibiotic which used for stimulation of blood haemoglobin production and so helps in cases of anaemia (Amit and Shailandra, 2006 ; Brooks, 2008). There are highly potassium rate in banana fruit, so, it can be used for blood Pressure. Also, it having low rate of salt, making it the perfect food for helping the beat of blood pressure (Debabandya et al., 2010). Bananas used for depression because of containing tryptophan protein which utilizing by the body converts it into serotonin known for making body relax and have important role in an improvement of the mood , creating happier feeling (Girish and Satish, 2008). Peel is a waste product of banana; it has medicinal properties (Imam and Akter 2011, Chabuck and *et al.*, 2013, Shadma *et al*., 2014). Many researchers were studied about the antimicrobial activity of banana peel against various Gram-positive and Gram-negative bacteria. Bioactive compound such as flavonoids, terpenoids, tannins, alkaloids and glycosides are found in banana peel. These compounds were widely used in pharmaceutical fields, especially as an antidiabetic, antioxidant, antibiotic and anti-inflammatory (Chabuck *et al.*, 2013). Lemon is an important medicinal plant, It is used mainly for its high alkaloids, which are playing an important role as an antibacterial agent and antitumor activities. Bioactive compounds such as polyphenols, the most important being vitamin C (or ascorbic acid) were found in crude

extracts of different parts of Lemon (flower, leaves, stem, juice, peel and root) which having activities against pathogenic bacterial strains . The fiber of citrus fruit also contains, citrus flavonoids which have a widely spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities (Kawaii *et al*., 2000; Burt, 2004; Ortuno *et al*., 2006). In different countries in the world, lemon juice is used as sanitizers to remove food borne pathogens from fresh vegetables, fruits, and fish (Sengun and Karapinar, 2004; Tomotake *et al*., 2006), lemon juice used in the treatment of wound infections, dysentery, diarrhea , typhoid fever, urinary tract infection and arthritis , so it can be taken instead of chemical medicines. Freshly squeezed lemon juice diluted with water or mixed with honey was believed to be potent against bacterial diseases (Kafaru, 1994).

MATERIALS AND METHODS

Microbial Isolates: Different 4 clinical microbial isolates 2 Gram positive (*Staphylococcus aureus, Streptococcus mutans*), 2 Gram negative (*Klebsiella pneumoniae , pseudomonas aeroginosa*) were collected and identified by using conventional biochemical tests and cultivated in pure culture (isolates bank) at microbiological laboratory/college of applied biotechnology/ AL Nahrain university.

P**lant collection and preparation of extracts:** Fresh bananas and lemon were obtained from the local market at Baghdad City, Iraq, 2017. Banana peels were firstly chopped and were kept in 70% isopropyl alcohol. Then, the entire mixture was homogenized in blender, this homogenized mixture or slurry was left at room temperature for about 48 h. As the reaction continued, the yellow transparent liquid turned to amber and later to an opaque black liquid that means as the indicator for completion of the reaction. After completion, the entire slurry was filtered by using Whatman filter paper and transferred into a falcon tube , then second filtered had made by using milipore filter unit (0.2 µm) to get banana peel extract (Edwards, 1999) . In this study isopropyl alcohol (70%) was used to dissolve more active compounds from the banana peel also used as a negative control solution.

The fresh lemon fruits were washed in running tap water and dried in laboratory, then they were peeled and the juice was squeezed out using a plastic juice extractor and put in sterile container, then the extracted fruit juice was subjected to double filtration with Whatman filter paper (to remove the seeds and other tissues) and 0.45µm membrane filter (Sigma) respectively and used freshly as crude with concentration 100 %.

Determination of antimicrobial activity: Loopfull growths from bacterial isolates were inoculated into nutrient broth incubated at 37 °C for 18 hours. The bacterial suspensions were diluted with normal saline. Adjust the turbidity and compare with standard tube(McFarland number 0.5) to yield a uniform suspension containing 1.5×108CFU / ml. Dip cotton swab into adjustment suspension and streak the Mueller-Hinton agar surface of plates three times by rotating the plate approximately 60º between streaking to ensure even distribution of the inoculums, the inoculated plates were placed at room temperature for 10min to allow absorption of excess moisture (Atlas *et al*., 1995).

Media were cut into well (5mm diameter) by cork borer (the wells were arranged so as to avoid the development of overlapping of inhibition zones) and add 0.1 ml (100 µl) of banana, lemon extracts and mixture (50 µl banana and 50 µl lemon) separately. Also, some plates are used as standard (antibiotic susceptibility test) as a positive control by used types of antibiotics (Ampicillin, Ciprofloxacin, Imipenem, Tetracycline) against Gram positive and negative bacteria.

The plates were incubated at 37 °C for overnight. After incubation, inhibition zones were measured by ruler also and the results were compared with the standards as in NCCLs, (2002).

RESULTS AND DISCUSSION

Results of antimicrobial activity of alcoholic banana peel extracts against Gram positive and negative isolates by the agar well diffusion method were shown on Table(1) and figure (1) , revealing no activity of banana peel extract on tested Gram positive and *Klebsiella pneumoniae* as in figure (1; B,C,D) but have effected only on *pseudomonas aeroginosa* as in figure (1; A).

Table (1): The antibacterial activities of alcoholic banana peel extract against pathogenic bacteria.

Bacterial isolates	Diameter of inhibition zone (mm)
Staphylococcus aureus	-
Streptococcus mutans	-
Klebsiella pneumonia	-
pseudomonas aeroginosa	12

Whereas, lemon juice extracts have good inhibitory effect on all tested bacteria shown on (Table 2) and figure (1) , also the mixture between the two extracts (banana and lemon juice) with equal volumes(50 µ from each one) in agar well , show high inhibitory effect on all tested pathogenic bacteria (Table 3) and figure (1) due to synergistic effect between them and secondary metabolite in lemon mainly for its alkaloids and having citrus flavonoids which have a large spectrum of biological activity including antibacterial , these active compounds have highly influence may induce the synergistic effect(mixture) between alcoholic banana peel and lemon juice extracts against bacteria .

These results were agreement with those obtained by Ahmad and Beg, (2001) investigated the alcoholic

extracts of *M. paradisiaca* banana fruit peel revealed activity against *Pseudomonas aeroginosa* (Gram-negative).

Malachy *et al*., (2015) who reported the antibacterial activities of lemon juice extract with concentration (100%) against Gram positive (*Staphylococcus aureus*) and negative bacteria (*pseudomonas aeroginosa*) with inhibition zone 19mm, 20mm respectively, that agreement with our results nearly, (Table 2). Hindi and Chabuck, (2013) also, studied about antimicrobial activity of lemon juice extract against pathogenic bacteia (*Staphylococcus aureus, Klebsiella pneumoniae*).

Zahra (2010), also investigated the antibacterial effects of crude of lemon juice (con. 100%) on Gram negative growth (*Klebsiella pneumoniae*) with zone of inhibition 15mm , which approach from our results (Table 2).

Table (2): The antibacterial activities of lemon juice extract against pathogenic bacteria.

Bacterial isolates	Diameter of inhibition zone (mm)
Staphylococcus aureus	15
Streptococcus mutans	8
Klebsiella pneumonia	10
pseudomonas aeroginosa	13

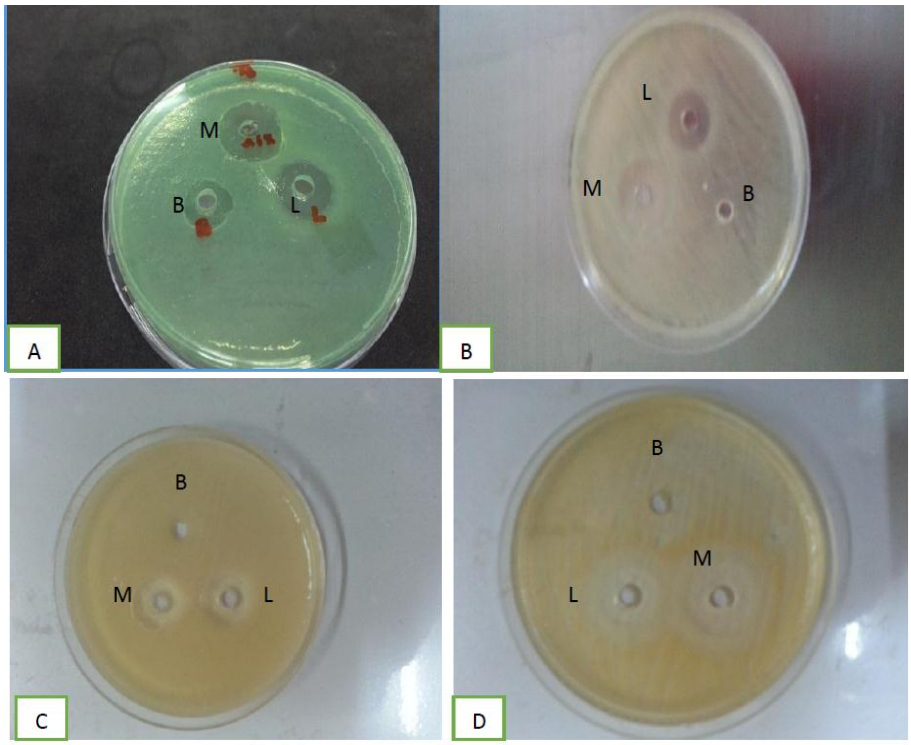

Figure (1): Antibacterial activity of banana peel extract and lemon juice extract and mixture : A *(pseudomonas aeroginosa*) , B *(Klebsiella pneumoniae*) , C *(Streptococcus mutans*) , D *(Staphylococcus aureus*). (L: Limon, B: Banana, M: Mixture between them).

Table (3): The antibacterial activities of mixture between lemon juice and alcoholic banana peel extracts against pathogenic bacteria.

Bacterial isolates	Diameter of inhibition zone (mm)
Staphylococcus aureus	22
Streptococcus mutans	11
Klebsiella pneumonia	15
pseudomonas aeroginosa	20

The standard drug, Ampicillin, Ciprofloxacin, Imipenem, Tetracycline showed 24 mm inhibition zone against *Staphylococcus aureus*, 27 mm inhibition zone against *Streptococcus mutans*, 15 mm inhibition zone against *Klebsiella pneumoniae* and 20 mm inhibition zone against *pseudomonas aeroginosa*, (Table 4).

Table (4): The susceptibility of antibiotics against pathogenic bacteria.

Bacterial isolates	Antibiotics	Inhibition zone (mm)
Staphylococcus aureus	Tetracycline	24
Streptococcus mutans	Ampicillin	27
Klebsiella pneumonia	Ciprofloxacin	15
pseudomonas aeroginosa	Imipenem	20

CONCLUSION

Based on our results, it can be concluded that the crude of lemon juice extract revealed the broad spectrum of antimicrobial activity than banana peel extract on the

tested bacteria with good inhibitory effective against all of them. Alcoholic banana peel extract showed antimicrobial effect against only *pseudomonas aeroginosa* , while the mixture between them showed highly antibacterial activity than each extract alone . These plant extracts are natural, cheap, safe and can be replacement the synthetic medicines and antibiotics in treatment of diseases caused by these pathogens , because of increasing the drug resistant bacteria that endanger the public health, so the use of medicinal plants with antimicrobial activity needs efforts and more attention to arrest this trouble. Due to rapid increasing of antibiotic resistance in our country, because of bacterial adaptation against different types of antibiotics; different parts of medicinal plants were utilized as medicines. The problem of microbial resistance is growing every day, therefore, concerted efforts must be made to reduce this problem, such as controlling of the use of antibiotic, develop research about the genetic mechanisms of bacterial resistance and continuous studies to develop new synthetic or natural drugs.

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