Molecular Detection of Burkholderia cepacia and Comparative Study Between Medical Plant and Antibiotics against Bacteria Isolates from Cystic Fibrosis

Article *in* Indian Journal of Forensic Medicine and Toxicology · June 2021

CITATIONS
0

READS
89

1 author:

Angham Najah Al-Khafaji
Al-Furat Al-Awsat Technical University' Kufa Technical Institute
45 PUBLICATIONS **24** CITATIONS

SEE PROFILE

Molecular Detection of *Burkholderia cepacia* and Comparative Study Between Medical Plant and Antibiotics against Bacteria Isolates from Cystic Fibrosis

Angham Najah Al-Khafaji[1], Mohammed Subhi Mohammed[1]

[1]Lecturer Kufa Technical Institute, Al-Furat Al-Awsat Technical University./Iraq

Abstract

Background: One of the major deaths causes in the patients with cystic fibrosis (CF) is the impaired lung function. Lately, *Burkholderia cepacia* was considered as a significant opportunistic pathogen in such patients. Since the late 1970s, due to its increased isolation from CF patients, its ability to spread infections in the community of CF patients, its role in disrupting lung function, and its inherent resistance to multiple antibiotics. Those various factors are making the *Burkholderia cepacia* isolation one of the significant tasks in cystic fibrosis health-care environment.

Objective: To identify the method and the complete recA nucleotide sequence, and to compare the isolated bacteria from CF by medicinal plants and antibiotics.

Material and Methods: Bacterial identification of CF patients was carried out by standard microbiological methods and identification was carried out using biochemical tests Vitek2 compact system, and PCR identification to confirm the identification and evaluate the antibacterial activity in *Mentha* leaves and lemon peel extracts against pathogenic bacteria.

Results: Biochemical tests and gene expression confirmed that *Burkholderia cepacia* and *Pseudomonas* spp strains were isolated from 50 sputum samples. These results indicate that *Mentha* leaves extract is better than lemon peel extract, compares with antibiotics reveal the resistance of these bacteria.

Keywords: cystic fibrosis, Mentha, Lemon peel extract, PCR, Vitek2 compact system, antibiotics.

Introduction

The major mortality and morbidity cause in CF patients is the chronic infection related to lower respiratory tract. The mutations changing the function regarding cystic fibrosis transmembrane conductance regulator (CFTR) are impairing the chloride transport and resulting in milieu favoring colonization via bacteria, especially *P.aeruginosa* and *S.aureus*. Lately, one of the other microbes, *Burkholderia cepacia* was indicated as one of the common colonizers in CF patients [(1).] Various organisms were recognized for having a role in CF's airway infections in the last 10-15 years. More and more species were indicated as dominant pathogens in airway, also, various co-infecting organisms are currently recognized [(2).] In addition, the members regarding *Burkholderia cepacia* complex (Bcc) were especially related connected to life-threatening infections of the respiratory tract in the patients experiencing chronic granulomatous disease (CGD), also they were the major transmissible, virulent, and inherently-resistant microbes emerging as pathogens of the cystic fibrosis in the most recent decades [(3).] Due to the fact that there is high importance in identifying *Burkholderia cepacia* in cystic fibrosis patients, then the micro-biological diagnoses must be achieved with extreme accuracy. Yet, reliable identification as well as the isolation of *Burkholderia cepacia* were difficult and complicated [(4).]

The food industries and consumers are majorly focusing on using the natural antibacterial compounds in food [(5).] Citrus is a flowering plant in *Rutaceae* family, it is native to the sub-tropical and tropical Southeast

Asia regions. In addition, there is a distinctive fragrance related to citrus fruits, somewhat because of the limonoids and flavonoids existence in the fruit's peel. Those fruits were excellent flavonoids and vitamin C sources. Citrus by-products, if fully utilized, maybe the main source of phenolic compounds. In particular, the peel is one of the rich sources of natural flavonoids, also the phenols' content in the peel is high compared to that of edible part. According to reports, the overall phenol content in grapefruit, orange and lemon peels is 15% high compared to peeled fruits [6] [7]It is showing that the citrus varieties were taken into account, also they have a lot of secondary metabolite sources and can produce a wide range of biological activities. Furthermore, the medicinal plants were utilized throughout the history for treating human diseases due to the fact that they are containing chemical ingredients with therapeutic value.

Aim of this study.

- Study the molecular biology of bacteria.
- Study effect of medical plant against bacteria.
- Identification and Isolation of bacteria from Cystic fibrosis.
- Compare the effect between medical plants and antibiotics against bacteria.

Material and Methods

***Patient and samples: -**

A total of 50 specimens were collected from all admitted patients who were suspected to cystic fibrosis to AL- sadder medical city during from october to january month. Also, the clinical specimens have been split from sputum specimens which are collected from patients with different ages.

***Identification and Isolation**

The single colonies are isolated from the primary positive cultures and after that identified based on the criteria stated by [8]. The use of certain media including macConkey Agar and blood agar identified via morphological; biochemical tests, the automated VITEK-2 compact system (VITEK2 GN ID Kit) has been utilized in the bacterial Diagnosis (Bio Merienx – france).

***PCR Product Detection**

The amplified products of the PCR are analyzed (routinely) via electrophoresis in 2% (wt/vol) agarose gels (Life Technologies GIBCO BRL Products) which contains 40mM Tris buffer (pH=8) as well as 20mM acetate. The molecular size markers_100) bp ladder; Life Technologies GIBCO BRL Products) have been operated in parallel on all the gels. Resolved products of the DNA are stained with EtBr and then viewed under the ultraviolet light.

Preparing the template DNA from the sputum samples of the cystic fibrosis patients.

Liquefied sputum (1 ml) (sputolysin-treated) has been 10,000 to 10 minutes centrifuged. In a TE buffer of 0.2 ml, the resulting pellet is reused. Bacterial cells were split into liquid nitrogen by snap freezing for 3 minutes and heat at 100°C for 1 minute [9]. Three more times this step was repeated. For ensuring complete bacterial cell lysis, the samples have been treated with 150 µg lysozyme at a temperature of 37°C during 30 minutes. In addition, the DNA has been purified as indicated for the bacterial cultures following proteinase K therapy and precipitation with isopropanol.

PCR analysis

PCR analysis has been conducted with a thermal cycler of the DNA.

The *B. cepacia recA* (1040 bp) complex gene was amplified with BCR1 and BCR2 primers (Table 1). Targeting the locus of 5′ and 3′ ends of *recA* gene [10]. A total volume of 50 µl was performed with PCRs. 10mM of Tris-HCl (pH 8.30), KCl of 50mM, 200µM each of triphosphate deoxynucleoside samples (Amersham-Pharmacia Biotech, Little Chalfont, UK), 100nM each BCR1 (Sigma-Aldrich), 2,5U DNA polymerase AmpliTaq (Perkin-Elmer), also 100ng of the pure genomic DNA or 2µL of the sputum DNA. BCR2 (Sigma-Aldrich) In initial samples, samples were heated by 35 cycles at 97°C (37°F) at 96°C (1 minutes) and 56°C (1min) and 72ºC (1.5min) at 56°C (1.5 min) prior to the amplification of *recA* sequence. The final extension phase at a temperature of 72°C has been completed during 10 mins. The *recA*-based detection related to *B. cepacia* complex organisms in sputum.

Table1: Primers that have been utilized for the identification and detection of B. cepacia complex genomovars in cystic fibrosis sputum samples

Gene	Primer pair sequences (5' to 3')	Specificity
BCR 1	TGA CCG CCG AGA AGA GCA A	recA gene—B. cepacia complex
BCR 2	CTC TTC TTC GTC CAT CGC CTC	recA gene—B. cepacia complex

***Preparation of Lemon peel and mentha Leaves extracts:**

The fruits of lemon (citrus lemon) it has been getting of local markets has been taking peel limes and after that left to dry in dry and dark place, then, it has been crushed via electric grinder for the purpose of obtaining peel's powder. Powders of l0g have been subjected to steeping in cold distilled water of l00ml, then the mixture is kept at room temperature of two hours, filtered via a filter paper (what man no1). In addition, the filtrate will be evaporated in the oven for 3 days at a temperature of 80℃. After that, the dried powder was transferred to a sterile universal tube in the refrigerator to be used later. Whereas the *mentha* was prepare via dissolving a powder of 5g placed in distilled water of l00ml in conical flask, whereas the crude preparation has been kept overnight in room temperature at 2h, then subjected to centrifuging for 10mins at 3000rpm. Also, the supernatant that contains plant extract has been transferred after that to preweighed beaker and the extract has been concentrated via evaporating with the use of oven for 3 days at 80℃.

***Effect of Lemon peel and *mentha* extract on Bacteria: -**

The screening related to antimicrobial activities regarding each one of the crude aqueous lemon peels as well as mentha extracts on tested bacteria utilized in this work has been evaluated on the muller-Htinton Agar via utilizing well diffusion approach. Wells with diameter of 6mm and depth of 5mm are borer [11]. About 20ml regarding each one of the extracts has been inoculated onto wells made in spread plate culture related to each one of the microbial isolates. In addition, all plate is incubated overnight at 37c. Following incubation for 24hrs, each one of the extract has been indicated for Zone of inhibition of or isolates. Zone inhibition's diameters are measured via evaluating via a measuring sale in millimeter (mm).

Antimicrobial susceptibility testing: -

The bacteria isolates were tested for their sensitivity to a variety of chemotherapeutic agents by use disc diffusion approach. Also, the test has been done with the use of muler-Hinton agar through using antibiotics disc. The growth inhibition zones around each one of the antibiotic discs were evaluated to nearest millimeter. In addition, the zone's diameter associated with the susceptibility of the isolate as well as the drug's diffusion rate through the agar medium [12].

Results and Discussion

Fifty clinical isolates from cystic fibrosis were distributed as follow, *Burkholderia cepacia* 30(60%), *Pseudomonas oryzihabitans* 7(14%), *Pseudomonas aeruginosa* 13(26%), as show in Figure 1.

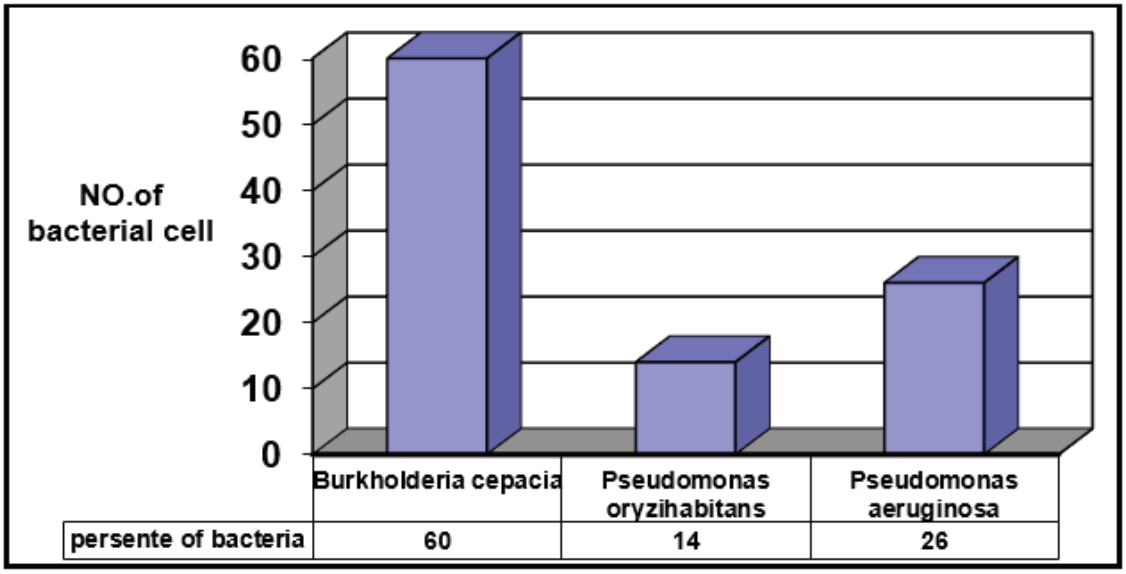

Figure 1: Distribution of Bacteria among clinical isolate of cystic fibrosis

Bacteria isolates have been identified on the basis of gram stain preliminary tests succeeded via biochemical characteristics of these isolates were studied and final identification with use VITEK2 compact system,

It isn't straight forward to identify cultured organisms obtained from the respiratory specimens in patients experiencing CF. The use of commercial systems, members of Bcc were mistaken as (amongst others) *B.gladioli Ralstonia pikettii, Pseudomonas* spp, *Alcaligenes* spp, *Flavobacterium* spp, *Stenotrophomonas maltophilia* and *Chryseobacterium* spp. The strains regarding such different species were mistaken as part of the *Burkholderia cepacia* complex [8].

recA-based PCR detection and identification of the ***B***. ***cepacia*** complex genomovars in the sputum.

Samples from patients experiencing CF which are infected with Bcc organisms have been analyzed with a view to investigating whether *B. cepacia* complex *recA* gene might successfully be enlarged from the preparations of the sputum DNA. *B. cepacia* genomovar was present in these sputum samples. BCR1 and BCR2 PCR analyzes demonstrate that Bcc organisms could be detected from sputum DNA directly via *recA* gene, which has successfully been amplified by all samples. Figure 2. shows a product of the *recA* gene amplified by sputum of Bcc complex genomovars infected patients. Because of the non-specific binding regarding primers, the amplified strips were clear and sharp. also, very reproducible was the PCR amplification of sputum DNA *recA* géne.

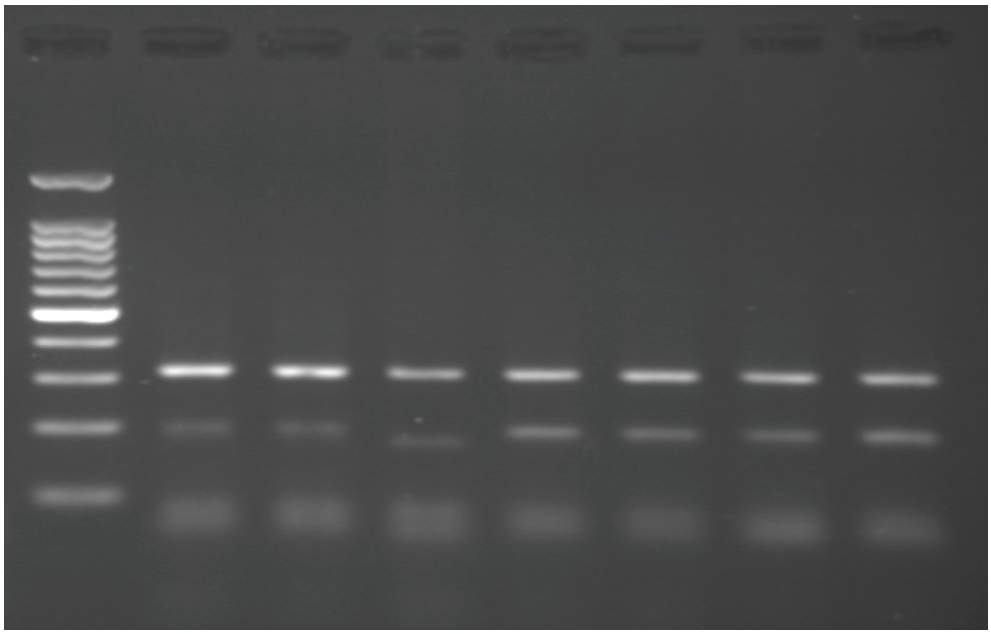

Figure2: PCR amplification of the 1kb *B. cepacia* complex *recA* gene from the cystic fibrosis patient sputum

The antimicrobial activity of the Lemon peel and *mentha* leaves extract in four concentrations (125,250,500,1000mg/ml) tested against bacteria isolate from cystic fibrosis has been evaluated via agar well diffusion approach. as show in Figure 3.

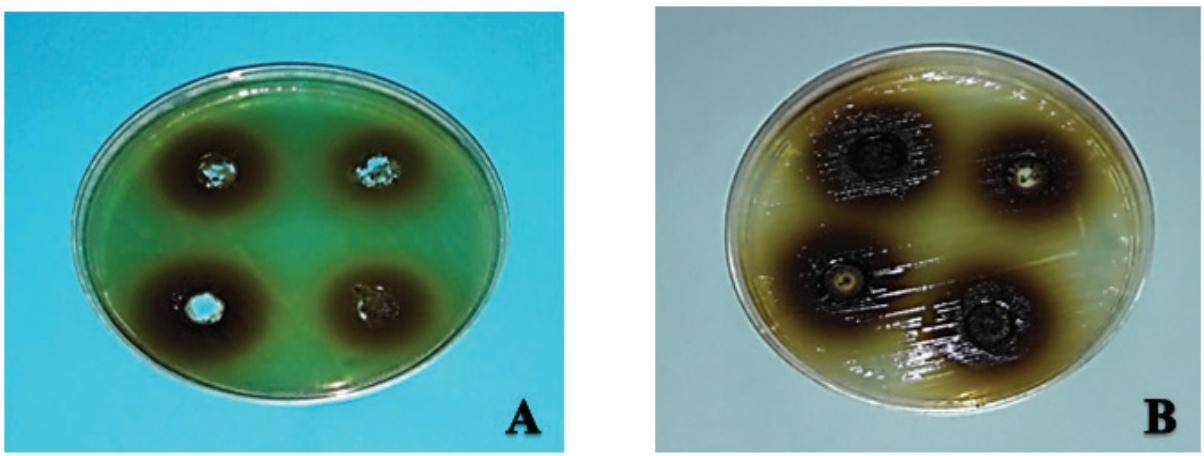

Figure 3: Antibacterial activity of medical plant at four concentrations against Test bacteria, A: Lemon peel extract, B: *Mentha leaves extract*

Result in Figure 4. shown the antibacterial activity related to lemon peel extracts in concentration (1000mg/ml) has good effect against all of the selected bacteria include *Burkholderia cepacia, Pseudomonas oryzihabitans, Pseudomonas aeruginosa*, the inhibition zone 25,28,25mm respectively. The observed antimicrobial activity is a result of the combination of more than single constituent. In addition, the different components could be acting in a synergistic manner [11]. The results of this work are in accordance with other studies indicating comparable antibacterial activity of peel extracts regarding the 3 fruits on chosen bacteria [13].

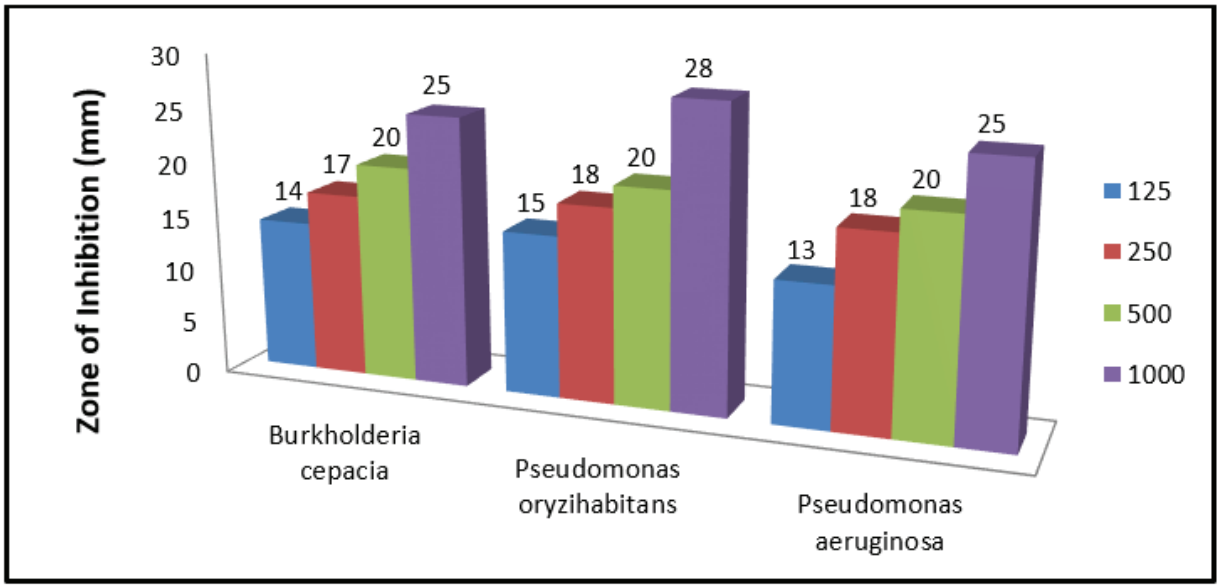

Figure4: Zone of inhibition (mm) of the extract of the Lemon peel of four concentrations (125,250,500,1000mg/ml) on selected bacteria isolate cystic fibrosis.

While the use *Mentha* leaves extract against tested bacteria is the high antibacterial activity than lemon peel extract as show inhibition zone 28,30,28mm respectively in concentration 1000mg/ml as in Figure 5.

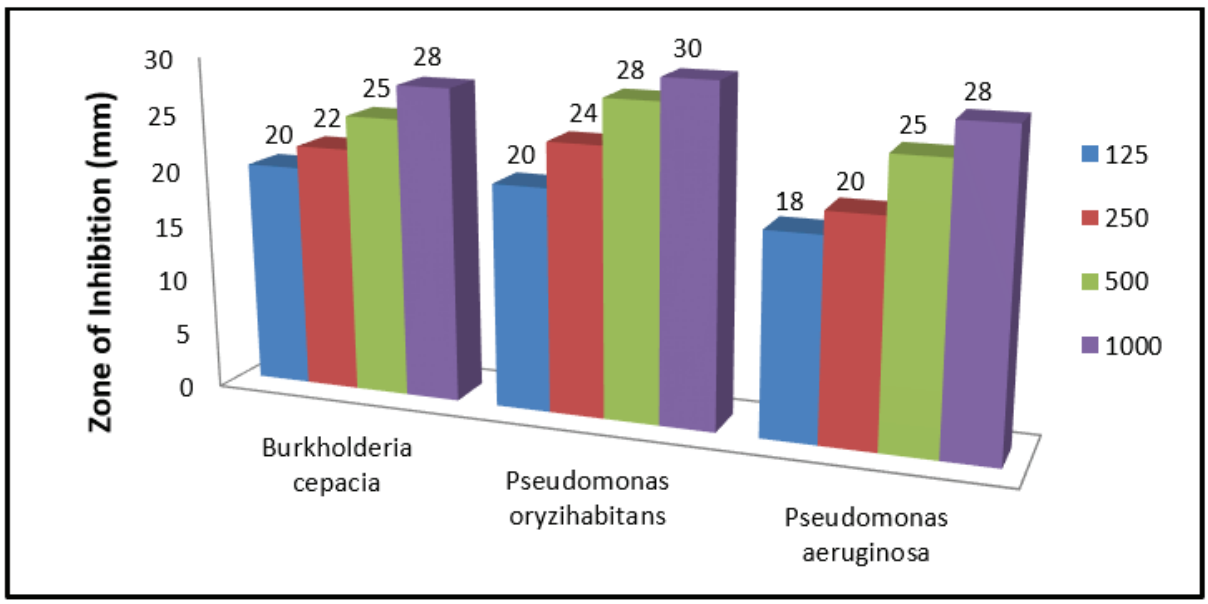

Figure 5: Zone of inhibition (mm) of *Mentha* leaves extract of four concentrations (125,250,500,1000mg/ml) on selected bacteria isolate cystic fibrosis.

The antibacterial activity has been indicated at different levels with the activity being strain as well as dose dependent. Also, the different crude extracts related to *Mentha piperita* indicated a considerable activity against every tested bacteria. Comparable to the results of this work, the biological activity related to *Mentha piperita* against pathogenic bacteria are indicated via (14). This result agrees with (15-17) on the effect of these plant on pathogenic bacteria isolate from clinical specimen. resemble with another study to absorved medical plant high effective against pathogenic bacteria (18).

Susceptibility of bacteria to 7 antibiotics via diffusion plate approaches utilizing paper disc method and the recorded data indicated that *Pseudomonas oryzihabitians* and *Burkholderia cepacia* were sensitive to Gentamicin, Tobramycin, Levofloxacin, whereas it was resistant to Ampicillin, Penicillin, Clarithromycin and Oxacillin.

Thus, the initial antibiotic therapies related to CF exacerbation was determined typically via the results regarding antibiotic susceptibility testing achieved on the isolates which are recovered from most-recent patient's culture [19]. The outer membrane regarding the gram negative bacteria shows a barrier to the penetration of various antibiotic molecules, also the periplasmic space containing enzymes, which have the ability to break down foreign molecules introduced from the outside; therefore, offering more greater resistance to them [20]. This result agree with [21] on the reveal resistance of different antibiotics on bacteria isolate cystic fibrosis. Similar to another study on the effect of antibiotics on pathogenic bacteria [22, 23].

Figure 6. shown the comparison in antimicrobial activity related to menthe leaves extract, lemon peel and Levofloxacin antibiotics against test bacteria with an inhibition zone diameter of Lemon peel 25,28,20mm respectively, while *Mentha* leaves extract 28,30,28mm respectively, in comparison with Levofloxacin as 20,22,18mm. On the other hand, Mentha leaves extract showed a lot antimicrobial activity in all tested bacteria than lemon peel extract and Levofloxacin antibiotics.

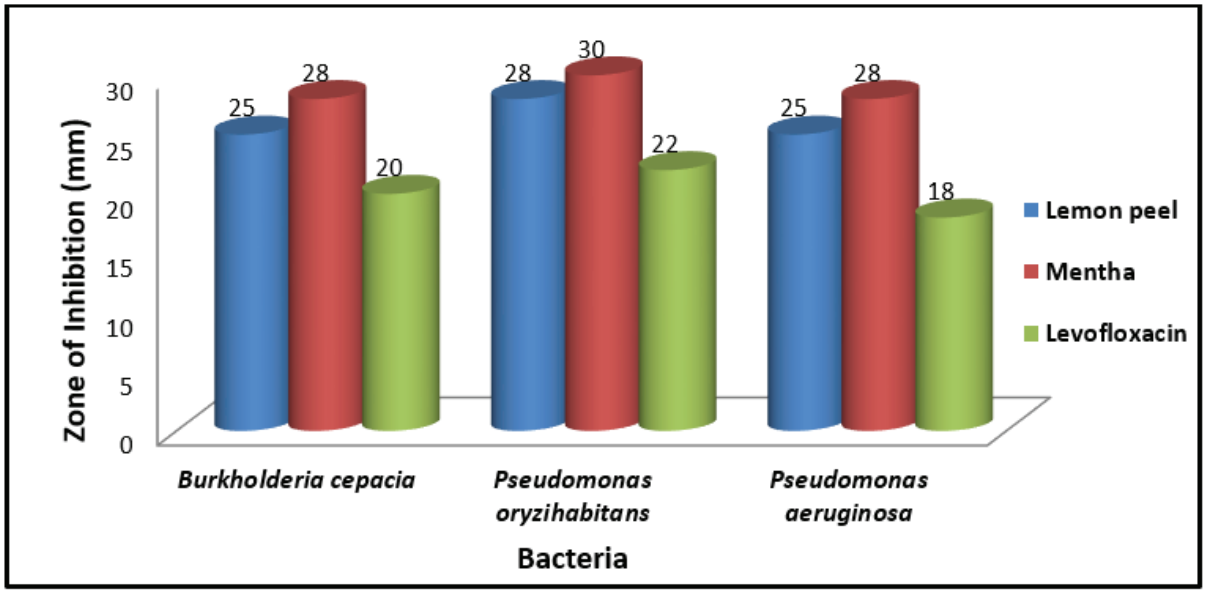

Figure 6: Comparison of medical plant and Levofloxacin antibiotics against bacteria isolate from cystic fibrosis.

Plants as well as plant products were extensively utilized since a long time for treating the medical problems. Various researches were conducted for extracting many natural products in terms of screening antimicrobial activity [24]. Similar to another study on the comparative effects of medicinal plants and antibiotisc on pathogenic microorganisms [25]. The varying sensitivity degree of bacterial strains could be because of the intrinsic tolerance regarding the bacterial, also the combination and the nature of phytocompounds existing in the extracts as indicated via [26]. The results of this study are comparable to [14] showing that the *Mentha piperita* compounds have potent antimicrobial activity, specifying that *Mentha piperita* leaf extracts must include effective active constituents, which are accountable to eliminate bacterial pathogens.

Conclusions

Infections in cystic fibrosis airway were poly-microbial and a lot of possibly-dominant emerging pathogens exist. It is significant that adequate works are conducted for identifying such organisms and determining the optimum antimicrobial treatments. In this work, the effect medical plant promising antimicrobial activity towards most-prevalent micro-organisms in CF, compare with antibiotics reveal most bacteria resistance of these.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

References

1. Lyczak JB, Cannon CL, Pier GB. Lung infections associated with cystic fibrosis. Clinical microbiology reviews. 2002;15(2):194-222.
2. Balfour-Lynn I, Elborn JS. Respiratory disease: infection. Cystic fibrosis. 2007:137-58.
3. Mahenthiralingam E, Coenye T, Chung JW, Speert DP, Govan JR, Taylor P, et al. Diagnostically and experimentally useful panel of strains from the Burkholderia cepacia complex. Journal of Clinical Microbiology. 2000;38(2):910-3.
4. Kiska DL, Kerr A, Jones MC, Caracciolo JA, Eskridge B, Jordan M, et al. Accuracy of four commercial systems for identification of Burkholderia cepacia and other gram-negative nonfermenting bacilli recovered from patients with cystic fibrosis. Journal of Clinical Microbiology. 1996;34(4):886-91.
5. Benelli P, Riehl CA, Smânia Jr A, Smânia EF, Ferreira SR. Bioactive extracts of orange (Citrus sinensis L. Osbeck) pomace obtained by SFE and low pressure techniques: Mathematical modeling and extract composition. The Journal of Supercritical Fluids. 2010;55(1):132-41.
6. Sawalha SM, Arráez-Román D, Segura-Carretero A, Fernández-Gutiérrez A. Quantification of main phenolic compounds in sweet and bitter orange peel using CE□MS/MS. Food Chemistry. 2009;116(2):567-74.
7. Ghasemi K, Ghasemi Y, Ebrahimzadeh MA. Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. Pak J Pharm Sci. 2009;22(3):277-81.
8. Wright R, Moore J, Shaw A, Dunbar K, Dodd M, Webb K, et al. Improved cultural detection of Burkholderia cepacia from sputum in patients with cystic fibrosis. Journal of clinical pathology. 2001;54(10):803-5.
9. Whitby PW, Dick HL, Campbell PW, Tullis DE, Matlow A, Stull TL. Comparison of culture and PCR for detection of Burkholderia cepacia in sputum samples of patients with cystic fibrosis. Journal of Clinical Microbiology. 1998;36(6):1642-5.
10. Mahenthiralingam E, Bischof J, Byrne SK, Radomski C, Davies JE, Av-Gay Y, et al. DNA-based diagnostic approaches for identification of Burkholderia cepacia complex, Burkholderia vietnamiensis, Burkholderia multivorans, Burkholderia stabilis, and Burkholderia cepacia genomovars I and III. Journal of Clinical Microbiology. 2000;38(9):3165-73.
11. Espina L, Somolinos M, Lorán S, Conchello P, García D, Pagán R. Chemical composition of commercial citrus fruit essential oils and evaluation of their antimicrobial activity acting alone or in combined processes. Food control. 2011;22(6):896-902.
12. Humphries RM, Ambler J, Mitchell SL, Castanheira M, Dingle T, Hindler JA, et al. CLSI methods development and standardization working group best practices for evaluation of antimicrobial susceptibility tests. Journal of clinical microbiology. 2018;56(4).
13. Rakholiya K, Kaneria M, Chanda S. Inhibition of microbial pathogens using fruit and vegetable peel extracts. International journal of food sciences and nutrition. 2014;65(6):733-9.
14. Baratta MT, Dorman HD, Deans SG, Figueiredo AC, Barroso JG, Ruberto G. Antimicrobial and antioxidant properties of some commercial essential oils. Flavour and fragrance journal. 1998;13(4):235-44.
15. Al-Sum BA, Al-Arfaj AA. Antimicrobial activity of the aqueous extract of mint plant. Sci J Clin Med. 2013;2(3):110-3.
16. Harjan A, Al-Khafaji AN. Study the effect of some plant extracts efficiency to reduce the pathogenesis

Candida albicans isolates from different body areas in the patients with diabetes II. Plant Archives. 2018;18(1):973-8.

17. Mohsen AH, Mohsen IH, Al-Khafaji AN. Compared between the efficiency of chemotherapy and alcoholic extract of plant leaves Melia azedarch L. in the growth of many fungi that cause Tinea capitis in humans in the laboratory. Journal of Pharmaceutical Sciences and Research. 2019;11(2):393-7.
18. Al-Khafaji AN. Comparison of Antimicrobial Activity of Olive Leaves Extracts and Apple Cider Vinegar against Bacterial isolates Obtained from Otitis Media. journal of kerbala university. 2018;14(3):68-76.
19. Ratjen FA. Cystic fibrosis: pathogenesis and future treatment strategies. Respiratory care. 2009;54(5):595-605.
20. Duffy CF, Power RF. Antioxidant and antimicrobial properties of some Chinese plant extracts. International journal of antimicrobial agents. 2001;17(6):527-9.
21. Ahmed Abdel Khalek AF, Mohamed Mahdy H, M Sharaf AE-M, A Hussein M, Rabie M. Isolation and Biochemical Identification of Pathogenic Burkholderia Cepacia from Human Sources and Related Reactivity to Different Antibiotics. 2013.
22. Al-Khafaji AN. Isolation and Identification of Methicillin Resistance Staphylococcus aureus and Detection their Ability to the Production of Virulence Factors. Journal of University of Babylon for Pure and Applied Sciences. 2018;26(8):100-11.
23. Al-Khafaji AN. Therapeutically and Synergism effect between Anastatica hierochuntica and antibiotics against Multidrug resistance bacteria isolate from Endometritis. Biochemical and Cellular Archives. 2018;18(2):2143-50.
24. Nitta T, Arai T, Takamatsu H, Inatomi Y, Murata H, Iinuma M, et al. Antibacterial activity of extracts prepared from tropical and subtropical plants on methicillin-resistant Staphylococcus aureus. Journal of Health Science. 2002;48(3):273-6.
25. Al-khafaji AN, Muhsin AH, Abdallah MT. Antifungal Activity of Crude and Phenolic Extract to Rice Crusts and Chemical Pesticide (Blitinute) in Inhibition of Fungi Isolate from Rice Seeds. Indian Journal of Forensic Medicine & Toxicology. 2020;14(2):1353.
26. Nanasombat S, Lohasupthawee P. Antibacterial activity of crude ethanolic extracts and essential oils of spices against Salmonellae and other enterobacteria. Current Applied Science and Technology. 2005;5(3):527-38.