Plant Archives Volume 20 No. 2, 2020 pp. 4815-4821 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF ANTIBACTERIAL ACTIVITY OF SOME PLANT EXTRACTS ON OPPORTUNISTIC BACTERIA

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Abstract

Staphylococcus aureus, *Pseudomonas aeruginosa* were consider as opportunistic organisms, in the case of immuno suppressed patients these caused diseases. these organisms resistant to multi-antibiotics so must use new therapy to depressed infection. Plant extracts have been used to control opportunistic diseases. Antimicrobial activity of two plant extracts were investigated against *S. aureus*, *P. aeruginosa* using agar disc diffusion technique.

Ethanol extracts of *Myrtus communis,* were potentially effective with variable efficiency against the tested bacterial strains at concentration of 100-150-200 mg/ml while extract of *Citrus aurantium* was not effective against *P. aeruginosa* at concentration of 100 mg/ml. *Citrus aurantium* extract was found to be effective with concentration of (200 mg/ml) against *P. aeruginosa* (clinical isolates) suppressing their growth with inhibition zones of 6 mm. The inhibitory effect of *Myrtus communis* extract started at 100mg/ml with inhibition zones of 2 and 3.16 mm against S. aureus and *P. aeruginosa* (clinical isolates), whilst 10.1 mm against *P. aeruginosa* (environmental isolates).

Inhibition zones at 150mg/ml of 4.13 and 4.16mm against *S. aureus and P. aeruginosa* (clinical isolates) while higher inhibition zone (12.16mm) was recorded in *P.aeruginosa* (environmental isolates). In concentration (200mm) of extracted plants of *Citrus aurantium* and *Myrtus communis*, the inhibition zone of *S. aureus* and *P. aeruginosa* (clinical isolates), *P. aeruginosa* (environmental isolates) were recorded (4.5,13.2,6.16) respectively. Inhibition with plant ethanolic extracts showed significant antibacterial activity against opportunistic pathogenic bacteria (*S. aureus* and *P. aeruginosa)*. Significant differences were observed in the treatment of all isolates with ethanolic plant extracts except for the clinical isolates of *P. aeruginosa*, where no significant differences were found for the absence of effect of *Citrus aurantium* extract at concentration (100 mg / ml) at $P<0.05$.

Key words: *Staphylococcus aureus, Pseudomonas aeruginosa*, plant extracts, antibacterial activity, disk diffusion.

Introduction

Opportunistic bacteria is established on two criterions: the receptive matter and the bacteria. In theory, if the receptive subject is normal, normal commensal organisms are not able to exceed the matter. This matter can only be contaminated by certain "undesirable" commensal microbial. Moreover, when normal person has a temporary decline in immune system, he may be pervaded by a number of organisms from the normal commensal flora, that consider as the opportunistic bacteria in the wide concept of the term.

Autoimmune and inflammatory diseases, all linked with non regulated immune responses have been raising dramatically during the past few decades. In new year's, immunological research has developed from a lymphoid tissue-centric view of the immune system to the understanding of tissue microenvironments as a essential determinant of immune responses. This area of research has led to the combination of the microbiota as an intrinsic regulator of all immune responses (Belkaid and Hand, 2014). *Staphylococcus aureus* is a gram-positive, round-shaped bacterium, establish in the upper respiratory tract and on the skin. It is positive for catalase and nitrate lowering and is a optional anaerobe that can grow without the requirement for oxygen (Masalha *et al*., 2001).

Generally, *S. aureus* consider a commensal of the human micro -biota, it can also become an opportunistic bacteria, being a prevalent cause of skin infections including furuncle, respiratory infections such as sinusitis and food poisoning. Clinical strains in some time promote infections by manufacturing virulence agents like strong protein toxins and the expression of a cell-surface protein

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that binds and inactivates antibodies. The development of antibiotic-resistant strains of *S. aureus* like methicillin-resistant *S. aureus* (MRSA) is a worldwide trouble in clinical medicine.

An evaluated 20% to 30% of the human population are prolong transporters of *S. aureus* (Kluytmans *et al*., 1997; Tong *et al*., 2015) was established as portion of the common skin flora, in the nostrils (Kluytmans *et al*., 1997). *S. aureus* is a considerable human pathogen that causes a wide range of clinical infections. It is a most important produce of bacteremia and infective endocarditis as well as osteoarticular, skin and soft tissue, pleuropulmonary and device-related infections (Tong *et al*., 2015) and normal found in the lower reproductive tract of women (Hoffman *et al*., 2012). *S. aureus* can cause pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia and sepsis. As yet *S.aureus* consider common reason of hospital-acquired infections and is often the cause of wound infections after surgery.

Pseudomonas aeruginosa is a complex gram-negative facultative anaerobe filled with a diversity of arsenals to operative, modify and damaging host defense mechanisms. The microbe is a common produce of nosocomial infections and an antibiotic-resistant priority pathogen. In the lung, *P. aeruginosa* disrupts upper and lower airway homeostasis by causing physical damage the epithelium and evading innate and adaptive immune responses (Curran *et al*., 2018).

The utilize of and investigation for drugs and dietary supplements derived from plants have accelerated in recent years. While 25 to 50% of present pharmaceuticals are derived from plants, none are used as antimicrobials. Conventional therapists have long used plants to block or healing infectious conditions, Western medicine is trying to duplicate their successes. Plants are rich in a wide diversity of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties (Cowan, 1999). In spite of the fact plant extracts have large possibility in the handling of infectious diseases caused by resistant a bacterium that is useful in biotechnology, but it is sudden that less than 5% plant types have been resolved for manufacture of antimicrobial components, whilst rests of the 95% of plants still need to be analyzed (de Lemos *et al*., 2004). Medicinal plants have historically confirmed their value as a source of molecules with therapeutic potential and at the present time still represent an important pool for the determination of novel drug leads. In the past decades, pharmaceutical manufacture converged mainly on libraries of synthetic compounds as drug discovery source (Atanasov *et al*., 2015).

Approximately 80% of the African population uses conventional plants to deal with health problems, basically because of their uncomplicated accessibility and affordability. It can be completed that medicinal plants have played and will continue to play important function in the administration of reproductive healthcare (Tsobou *et al*., 2016). The plant extracts used in traditional medicine have been developed and suggest to treat chronic and infectious diseases (Del Campo *et al*., 2007). Medicinal herbs practiced in traditional folk medicine in Pakistan have been screened for the presence of antibacterial activity. Local people in the Sudhnoti district of Pakistan share a rich practice of conventional medicine for the treatment of a diversity of diseases (Khan *et al*., 2018).

Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. The present investigation represents an introductory sieving on various plant extracts for the isolation and identification of biologically active components having antimicrobial activity. These compounds can be of significant importance in improving the shelf-life of food products because majority of these component are safe for human consumption and provide hurdle to the growth of food borne pathogens and damage bacteria. diverse studies have reported that medicinal plants manufacture a large number of secondary metabolites with antimicrobial effects on pathogens (Willett *et al*., 2004).

Myrtus communis L. is an evergreen aromatic plant. The major oil components of leaves of two origins at flowering stage were α-pinene (3.8-23.0%), 1,8-cineole (9.9-20.3%), limonene (5.5-17.8%), linalool (12.3-17.6%) and α-terpinyl acetate (1.8-7.0%). The leaf oil compositions at fruit ripening stage was highly similar to those of flowering stage as 1,8-cineole (24.0%), α-pinene (22.1%), limonene (17.6%), linalool (11.4%), linalyl acetate (4.5%), α-terpinyl acetate (2.2%) and geranyl acetate (1.2%) were main components. Major constituents of fruit oil were α-pinene (28.6%), 1,8-cineole (26.7%), limonene (18.0%), α-terpinyl acetate (5.4%), linalyl acetate (3.4%) and linalool (2.3%) (Pezhmanmehr *et al*., 2013).

Citrus aurantium L. (Rutaceae), usually recognized as bitter orange, possesses multiple therapeutic potentials. These biological credentials involve anticancer, antianxiety, antiobesity, antibacterial, antioxidant, pesticidal and antidiabetic activities. The essential oil of *C. aurantium* was described to display marked pharmacological effects

and great variation in chemical constitution determining on growing locations but mostly contained limonene, linalool and β-myrcene. Phytochemically, *C. aurantium* is rich in p-synephrine, an alkaloid and many health-giving secondary metabolites such as flavonoids (Suntar *et al*., 2018), *C. aurantium* is also used for the treatment of several ailments such as gastrointestinal disorders and obesity (Moraes *et al*., 2009). Fundamental oil of the leaves used as anxiolytic (Carvalho -Freitas and Costa, 2002). The major purpose of the current study was to assess the inhibitory activities crude extracts of selected medicinal plants against some human pathogens that cause urinary tract infections.

Materials and Methods

Isolation of microbial strains

Seventy-five of *S. aureus* were isolates collected from Patients with skin infection,and isolation Sixty-two of *P. aeruginosa* isolated from patients from Education Hospital in Hilla and isolation forty-eight of environmental isolates of *P.aeruginosa* from river in hilla, all isolates were planted on petri dishes.

Preparation Nutrient Agar

Prepare Nutrient Agar and Nutrient Broth for Subculture and growth of bacterial isolates by Suspended 28 g of nutrient agar powder in 1000 ml distilled water after that sterilized by autoclaving at 15 lbs. pressure (121°C) for 15 minutes.

The samples cultured into nutrient agar. The isolates were purified with three replicates per isolation.

Preparation of Cetrimide Agar

To the segregation and diagnosis of *P. aeruginosa* by using cetrimide Agar is a selective medium utilized for clinical and nonclinical samples and to detection the susceptibility of bacteria to biofilm formation (PerezI and Barth, 2011).

Inoculums preparation

The samples cultured into nutrient agar. The isolates were purified, then bacterial strain was subculture at 37°C in Nutrient agar for 24 hour with three replicates per isolation. bacterial strains were sub cultured at 37°C in nutrient agar slants. The bacterial growth was cropped using 5 ml of sterile saline water, its absorbance was set at 580 um and watered to get fertile cell numeration of 107 CFU/ml using spectrophotometer.

Collection of Plant Materials

Collected the leaves of *Myrtus communis, Citrus aurantium* and left the leaves under the sun for two days to become dry and then ground the leaves and weight of 20 g of powder and kept in a glass container until the preparation of alcohol extract (Joy *et al*., 1998).

Preparation of plant extracts

Nearly fifteen grams of methanol leaves extract of *Myrtus communis, Citrus aurantium*. flour was saturated in 30 ml methanol for ten hours in a rotatory shaker. Whitman No.1 filter paper was used to separate the extract of plant. The filtrates were used for further phytochemical analysis. It was again filtered through sodium sulphate in order to remove the traces of moisture (Hameed *et al*., 2015; Hussein *et al*., 2015).

Antibacterial activity of plants extracts

The disk diffusion procedure is utilized to estimate antimicrobial activity of plant extracts. The plant extract remains (50 mg) were re-melted in 2.5 ml of ethanol, infertile through Millipore filter (0.22 μm). 20 ml of nutrient agar medium was poured into sterile Petri dishes then inoculated with bacterial suspension. The microbial growth inhibitory potential of the plant extracts was determined by using agar disc diffusion method (Balouiri *et al*., 2016).

One microorganism of each strain was selected to determine the antimicrobial activity of plant extracts. The dishes were preserved in the refrigerator at 5°C for 2 hour to allow plant extracts spread then incubated at 37°C for 24 h with sterile petri dish was contain culture media and bacterial suspension without plant extract utilized as control to compared with sterile petri dishes contain bacterial suspension with plant extracts. The presence of inhibition zones were measured by standard ruler, registered as significant for antibacterial activity.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) and differences among the means were determined for significance at $P < 0.05$, using Duncan's multiple range test (by SPSS software) Version 9.1.

Results and Discussion

In this study, two plant species were studied to estimate their antibacterial activity against opportunistic bacteria consist of one strain of gram positive bacteria (*S. aureus*) and one strain of gram negative bacteria (*P. aeruginosa*) using disc diffusion method. to estimation of antibacterial activity of these plant extracts was registered in tables 1, 2 and explained in fig. 7. This study revealed that leaf of *Myrtus communis*, *Citrus aurantium* have very high bactericidal action on the common clinical and environmental isolates tested during this study. The isolates examined were *P. aeruginosa, S. aureus*. Results of antimicrobial activity of the two plant extracts

Table 1: Effect of *Myrtus communis* ethanol extract against *S. aureus, P. aeruginosa* (clinical), *P. aeruginosa* (environmental).

Inhibition zones (mm)			Concertation mg/ml	Plant extract
***Pseudomona aeruginosa* (clinical)**	***Pseudomonas aeruginosa* (environmental)**	***Staphylococcus aureus***		
3.16	10.1	2	100	*Myrtus communis*
4.16	12.16	4.13	150	
6.16	13.2	4.5	200	
Data are means of three replicates (n = 3)				

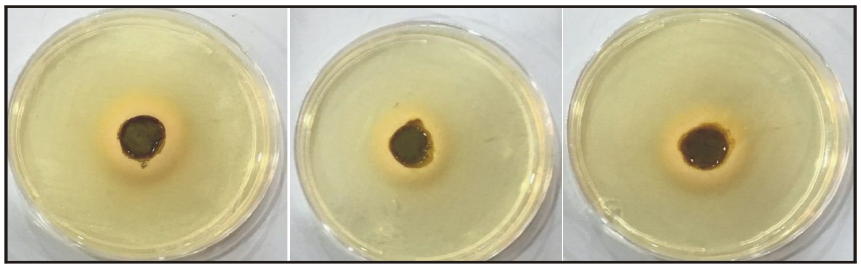

Fig. 1: *S. aureus* treatment with 100, 150, 200 mg/ml of *Mytrus communis*.

Table 2: Effect of *Citrus aurantium* ethanol extract against *S. aureus, P. aeruginosa* (clinical), *P. aeruginosa* (environmental).

Inhibition zones (mm)			Concertation mg/ml	Plant extract
***Pseudomona aeruginosa* (clinical)**	***Pseudomonas aeruginosa* (environmental)**	***Staphylococcus aureus***		
4.2	0.0	3.4	100	*Citrus aurantium*
4.8	2.1	4.16	150	
6	2.3	4.76	200	
Data are means of three replicates (n = 3)				

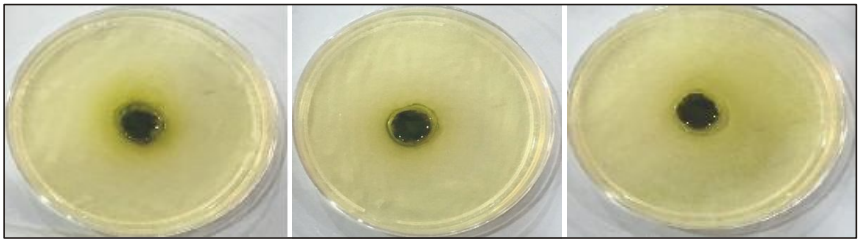

Fig. 2: *S. aurues* treatment with 100, 150, 200 mg/ml of *Citrus aurantium*

can suggested that *S. aureus* and *P. aeruginosa* were the most sensitive strains to the extracted plants respectively.

The inhibitory effect of *Myrtus communis* extract started at 100mg/ml with inhibition zones of 2 and 3.16 against S. aureus and *P. aeruginosa* (clinical isolates), whilst 10.1 mm against *P.aeruginosa* (environmental isolates). Inhibition zones at 150mg/ml of 4.13 and 4.16mm against *S. aureus and P. aeruginosa* (clinical isolates) while higher inhibition zone (12.16mm) was recorded in *P. aeruginosa* (environmental isolates). In concentration (200mm) of extracted plants, the inhibition zone of *S. aureus* and *P. aeruginosa* (clinical isolates), *P.aeruginosa* (environmental isolates) were recorded (4.5, 13.2, 6.16) respectively.

Our study was proved that the highest inhibition zone at concentration 200mg/ml at 13.2 against *P. aeruginosa* (environmental isolates). Extract of *Citrus aurantium* repressed bacterial growth of these strains of *S. aureus and P. aeruginosa* at concentration of 100 mg/ml with inhibition zones of 3.4 and 4.2 mm respectively, while *P.aeruginosa* was not repressed by *Citrus aurantium* at this concentration of extracted plant. At concentration 150mg/ml of 4.16 and 4.8 mm against *S. aureus* and *P. aeruginosa* (clinical isolates), in case of *P.aeruginosa* (environmental isolates) was depressed by *Citrus aurantium* for 2.1mm of inhibition zone. Whilst the higher inhibitory zone in *P.aeruginosa* (clinical isolates) 6mm at 200mg/ml concentration of extracted plant of *Citrus aurantium*.

S.aureus and *P.aeruginos* (environmental isolates) were defected at 200 mg/ml concentration of extract ethanol of Citrus aurantium with 4.76 and 2.3mm respectively of inhibition zone. The present study shows that the ethanol extracted of *Myrtus communis* t is the most effective on the bacteria strains and *P.aeruginosa* (environmental isolates) are most likely to be suppressed with two plant extracts (tables 1, 2 and fig. 1, 2, 3, 4, 5, 6).

The susceptibility of these organisms to these extracts explains their use in natural medicine for the treatment of infections such as dysentery, sore throat, cough and wound. The extracts were shown to exhibit a broad spectrum of antimicrobial property against the tested organisms. *S.aureus* was moderately susceptible to the extracts (*Myrtus communis, Citrus aurantium* extracted ethanol of leaf) while *P.aeruginosa* (environmental isolates) have higher susceptible to plant extracts. As a result, we strongly suggest that further investigation should be carried out on the effect of storage and other physical and environmental factors on the antimicrobial activity of the extracts.

Table 3: Statistical analysis using analysis of variance (ANOVA) and differences among the means were determined for significance at $P < 0.05$.

Concentration of *Myrtus communis* mg/ml	Mean and S.E. of *S. aureus*	Mean and S.E. of *P. aeruginosa* (clinical)	Mean and S.E. of *P. aeruginosa* (environmental)
100	2.06 ± 0.05	10.06 ± 0.15	3.05 ± 0.09
150	4.07 ± 0.06	12.05 ± 0.09	4.10 ± 0.09
200	4.3 ± 0.2	13.1 ± 0.1	6.09 ± 0.08

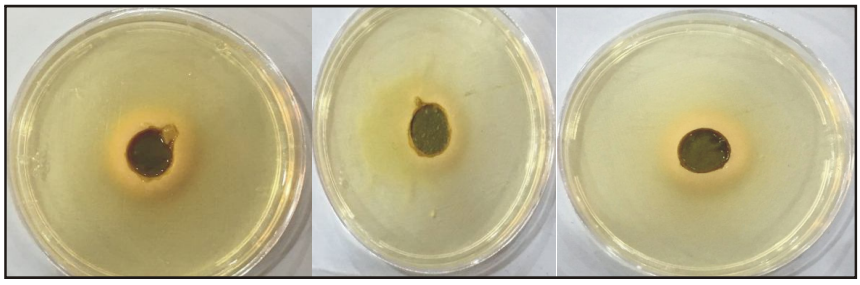

Fig. 3: Clinical isolates of *P.aureuginosa* that treatment with 100, 150, 200 mg/ml of *Mytrus communis*.

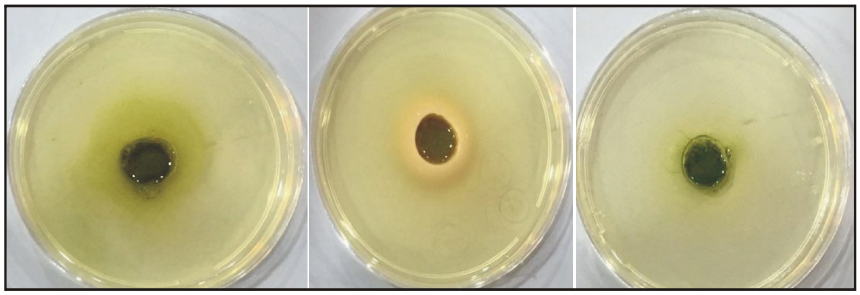

Fig. 4: Clinical isolates of *P.aureuginosa* that treatment with 100, 150, 200 mg/ml of *Citrus aurantium*.

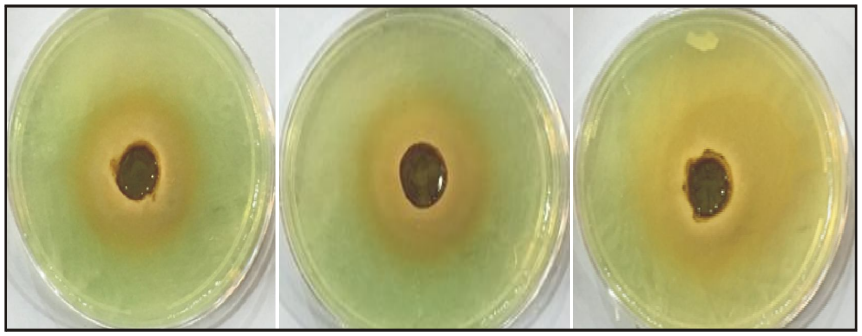

Fig. 5: Environmental isolates of *P.aureuginosa* that treatment with 100, 150, 200 mg/ml of *Mytrus communis*.

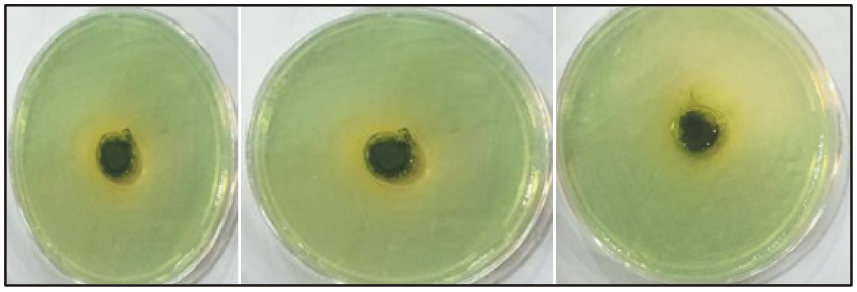

Fig. 6: Environmental isolates of *P.aureuginosa* that treatment with 100, 150, 200 mg/ml of *Citrus aurantium*.

Bacterial strains included in this study were chosen for their importance in infection by opportunistic bacteria. *S. aureus* considered as the one of the most common source of food borne disease while *P. aeruginosa* produce toxins and other metabolites that induce human gastroenteritis diseases. *Myrtus communis* extract suppressing microbial growth of all tested bacterial strains followed by extract of *Citrus aurantium* which appear to be weak effective against *P. aeruginosa* (environmental isolates) in contrast with *Myrtus communis* effective. The results of the current study are consistent with other researchers were proved the efficiency of ethanol extracts for *Myrtus communis* as antibiotic against *S. aureus and P. aeruginosa* in contrast with *Citrus aurantium* that appeared moderate effective (Mostafa *et al*., 2018).

On the other hand, in current study *Citrus aurantium* extract was found to be effective with concentration of (200 mg/ml) against *P. aeruginosa* (clinical isolates) suppressing their growth with inhibition zones of 6 mm. These results are in accordance with other investigation (Mahfuzul-Hoque *et al*., 2008). The present study suggested that plant extracts which proved to be potentially effective can be used as natural preservatives to control food poisoning diseases and preserve food avoiding application of health hazards of chemical preservatives.

So plant extracts inhabited a big place in diseases therapy and car for human health because, they include many energetic substances that can be utilized in the field of pharmaceutical producing from natural materials (Mohammed *et al*., 2017; Hussein *et al*., 2019). Statistical analysis of data were analyzed using analysis of variance (ANOVA), Significant differences were observed in the treatment of all isolates with ethanolic plant extracts except for the clinical

Table 4: Statistical analysis using analysis of variance (ANOVA) and differences among the means were determined for significance at $P < 0.05$.

Concentration of *Citrus aurantium*	Mean and S.E. of *S. aureus*	Mean and S.E. of *P. aeruginosa* (clinical)	Mean and S.E. of *P. aeruginosa* (environmental)
100	3.26±0.23	0.0±0.0	4.03±0.06
150	0.0±0.0	12.05±0.09	4.10±0.09
200	4.07±0.08	2.02±0.06	4.36±0.37

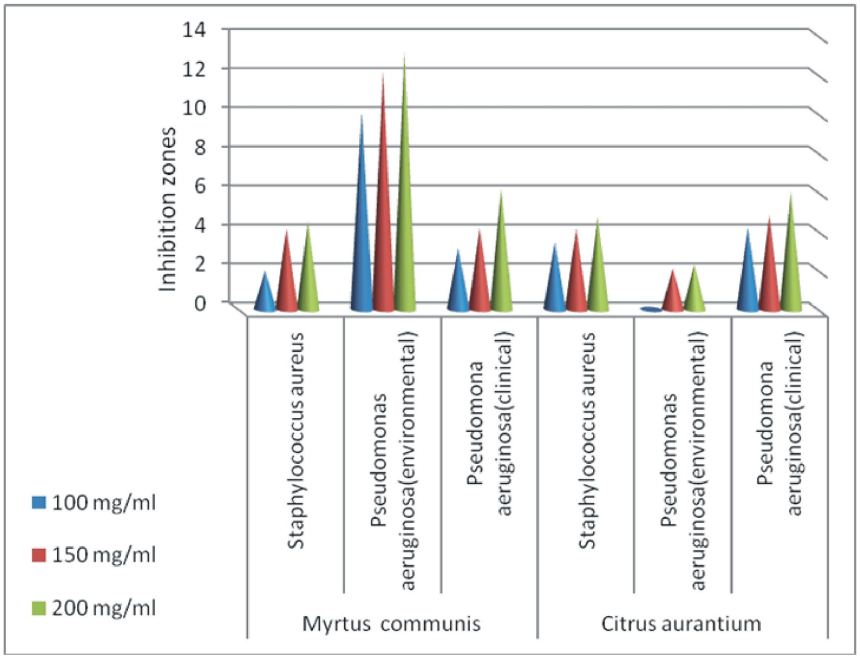

Fig. 7: Inhibition zones of *Myrtus communis*, *Citrus aurantium* ethanol extract against *S. aureus, P. aeruginosa* (clinical), *P. aeruginosa* (environmental)..

isolates of *P. aeruginosa*, where no significant differences were found for the absence of effect of Citrus aurantium extract at concentration (100 mg / ml) at $P < 0.05$. (Tables 3 and 4).

Conclusion

Our results offer that ethanol extracts for leaf of plant have ability for using as antibiotics on prevalent bacteria isolates that reason infection in our society. Antibiotics that can be created from these extracts would be helpful to reduced diseases by using natural antibiotics without side effects of this antibiotics.

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