

Research Article**Compared Anti bacterial and anti yeast effect of *Citrus sinensis* and *Musa sapientum* with the antibiotic penicillin on two pathogenic agent**Nazereh Hashempour^{1*}, Mohamad Hosein Sadeghi Zali², Saber Yousefi³, Amin Hashempour¹¹Young Researcher and Elites club, Islamic Azad University, Urmia Branch, Urmia Iran²Department of Bacteriology, Veterinary Faculty, Islamic Azad University, Urmia Branch, Urmia, Iran³Department of Microbiology, Virology and Immunology, Urmia University of Medical Sciences, Urmia, Iran***Corresponding author**

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Abstract: The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine. The aim of this study Compared antibacterial and anti yeast effect of *Citrus sinensis* And *Musa sapientum* with the antibiotic penicillin on two pathogenic agents. This study with MIC and MBC method was done. 2 agent of pathogen are *Salmonella typhimurium* and *Candida albicans*. In this study it can be concluded that effect of *Citrus sinensis* more than *Musa sapientum* and antibiotic Penicillin on *Salmonella typhimurium* and *Candida albicans*. Anti bacterial and anti yeast effect of extracts and antibiotic can described respectively as (a) *Citrus sinensis*, (b) *Musa sapientum* and (c) Antibiotic Penicillin. In MBC method final clear well was cultured on Mueller-Hinton agar and show growth.

Keywords: antibacterial, anti yeast, *Citrus sinensis*, *Musa sapientum*, Penicillin, Pathogen

INTRODUCTION

In the recent decades, antimicrobial plant products have gained a special attention because of increase resistance to antibiotics acquired of some microorganisms [1, 2]. Antibacterial activity is the ability of a substance to inhibit or kill bacterial cells. Different types of antibiotics and chemotherapeutic agents are being used in the treatment of one form of disease or the other. Most of these antibiotics were originally derived from micro-organisms while the chemotherapeutic agents are from plants. However, nowadays these antibiotics and chemotherapeutic agents are obtained by various synthetic processes [3, 4]. Infectious diseases represent a serious health problem today and account for one third of all deaths worldwide. Antimicrobials of plant origin have enormous therapeutic potential as they are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [5, 6].

The increase in prevalence of multiple drug resistance has slowed down the development of new synthetic antimicrobial drugs, and has necessitated the

search for new antimicrobials from alternative sources. Natural compounds are a source of numerous therapeutic agents. Recent progress to discover drugs from natural sources has resulted in compounds that are being developed to treat cancer, resistant bacteria and viruses and immunosuppressive disorders [7]. The aim of this study is compared antibacterial effect of 2 medicinal plants with penicillin antibiotics on 2 pathogenic agents.

MATERIAL AND METHODS**Collection of plant material**

Citrus sinensis was collected from Ramsar city in Mazandaran province, Iran on February 2014 And *Musa sapientum* bought from herbal plant bazaar in Urmia city center on April 2014.

Culture and Maintenance of microorganisms

Bacteria and Yeast was prepared from Iranian Research Organization for Science and Technology (IROST). After preparation this agent was sub cultured. Yeast was cultured on Saburd dextrose Agar media and salmonella was cultured on Mueller-Hinton Agar.

Table 1: characteristics of Bacteria and Yeast

SL. No.	Name	Type	PTCC No
1.	<i>Salmonella typhimurium</i>	Gram Negative bacteria	PTCC1609
2.	<i>Candida albicans</i>	Yeast	PTCC5027

Preparation of plant extract

After collecting plants peeling and dry was done. After drying, water extracting with maceration method was done. 5g from dry matter was added to 100cc sterile water. After 24 hours was passed through the filter. That extract had 5% concentration.

Microbiological screening

Antimicrobial activities of different extracts were evaluated by the Minimum inhibitory concentration (MIC) [8].

Media Preparation and Its Sterilization

For the wells we prepared Mueller-Hinton broth media. All the media prepared were then sterilized by autoclaving the media at (121°C) for 20 min.

Minimum Inhibitory concentration

The minimum inhibitory concentration (MIC) is defined as the lowest concentration able to inhibit any visible bacterial growth on the culture plates. It was determined from readings on the culture plates after incubation. The tube dilution method and agar dilution methods are most commonly employed methods. Serial dilutions are made of the products in bacterial and yeast growth media. The test organisms are then added to the dilutions of the products, incubated, and scored for growth. It is a standard procedure assay for antimicrobials [16].

MIC is important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. It is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism [16].

Clinically, the MIC is used not only to determine the amount of antibiotic that the patient will receive but also the type of antibiotic used that in turn

lowers the opportunity for microbial resistance to specific antimicrobial agents [16].

Test for antibacterial activity

The antibacterial assay was carried out by micro dilution method in order to determine the antibacterial activity of compounds tested against the pathogenic bacteria. The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0 X 10⁷ CFU/ml. All experiments were performed in duplicate and repeated four times [16].

Determination of MIC

The minimum inhibitory concentrations (MIC) and MBC were performed by a serial dilution technique using 96-well microtiter plates. The different plant extracts Aqueous were taken (5/100 g/ml) and serial dilution of the extract with Mueller-Hinton broth medium for bacterial and yeast were used. The micro plates were incubated for 24 hours at 37 °C, respectively. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs [16].

Determination of MBC

The MBCs were determined by serial sub-cultivation of 2 µl into microtitre plates containing 100 µl of broth per well and further incubation for 24 hours. The lowest concentration with no visible growth was defined as the MBC. All experiments were performed in duplicate and repeated four times [16].

RESULTS

In the present investigation, the inhibitory effect of different extracts of *Citrus sinensis* and *Musa sapientum* with the antibiotic penicillin was evaluated against both yeast and bacteria strains. The antimicrobial activity was determined using agar well diffusion method. The activity was quantitatively assessed on the basis of inhibition zone and their activity index was also calculated along with minimum inhibitory concentration (MIC).

Table 2: Effect of *Citrus sinensis* on *Salmonella typhimurium* and *Candida albicans* (MIC)

		1000	500	250	125	62.5	31.25	15.62	7.812
<i>Salmonella typhimurium</i>	Rep1	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth
	Rep2	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth
	Rep3	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth
	Rep4	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth
<i>Candida albicans</i>	Rep1	No growth	No growth	No growth	growth	growth	growth	growth	growth
	Rep2	No growth	No growth	No growth	growth	growth	growth	growth	growth
	Rep3	No growth	No growth	No growth	growth	growth	growth	growth	growth
	Rep4	No growth	No growth	No growth	growth	growth	growth	growth	growth

Table 3: Effect of *Musa sapientum* on *Salmonella typhimurium* and *Candida albicans* (MIC)

		1000	500	250	125	62.5	31.25	15.62	7.812
<i>Salmonella typhimurium</i>	Rep1	No growth	growth	No growth	No growth	No growth	No growth	No growth	No growth
	Rep2	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth
	Rep3	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth
	Rep4	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth
<i>Candida albicans</i>	Rep1	growth	growth	growth	growth	growth	growth	growth	growth
	Rep2	growth	growth	growth	growth	growth	growth	growth	growth
	Rep3	growth	growth	growth	growth	growth	growth	growth	growth
	Rep4	growth	growth	growth	growth	growth	growth	growth	growth

Table 4: Effect of antibiotic penicillin on *Salmonella typhimurium* and *Candida albicans* (MIC)

		1000	500	250	125	62.5	31.25	15.62	7.812
<i>Salmonella typhimurium</i>	Rep1	No growth	No growth	No growth	growth	growth	growth	growth	growth
	Rep2	No growth	No growth	No growth	growth	growth	growth	growth	growth
	Rep3	No growth	No growth	No growth	No growth	growth	growth	growth	growth
	Rep4	No growth	No growth	No growth	No growth	growth	growth	growth	growth
<i>Candida albicans</i>	Rep1	growth	growth	growth	growth	growth	growth	growth	growth
	Rep2	growth	growth	growth	growth	growth	growth	growth	growth
	Rep3	growth	growth	growth	growth	growth	growth	growth	growth
	Rep4	growth	growth	growth	growth	growth	growth	growth	growth

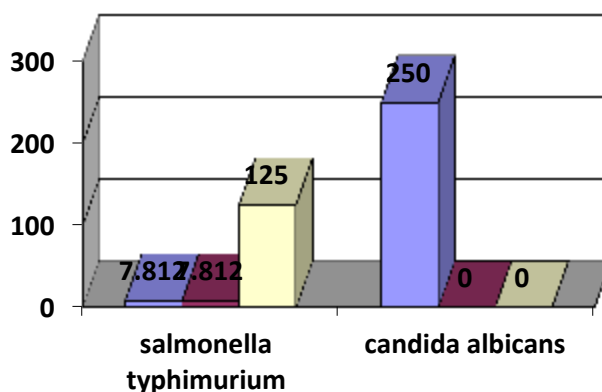


Fig. 1: MIC result of *Citrus sinensis*, *Musa sapientum* and antibiotic Penicillin on *Salmonella typhimurium* and *Candida albicans*

In this study it can be concluded that effect of *Citrus sinensis* more than *Musa sapientum* and antibiotic Penicillin on *Salmonella typhimurium* and *Candida albicans*. Anti bacterial and anti yeast effect of extracts and antibiotic can be described respectively (a)

Citrus sinensis, (b) *Musa sapientum* and (c) Antibiotic Penicillin.

In MBC method final clear well was cultured on Mueller-Hinton agar and show growth.

Table 5: Growth on concentration in final clear well

Sl. No.	Name of bacteria	<i>Citrus sinensis</i>	<i>Musa sapientum</i>	Penicillin
1.	<i>Salmonella typhimurium</i>	7.812/ no growth	7.812/ no growth	125/ growth
2.	<i>Candida albicans</i>	250/ growth	1000/ growth	1000/ growth

DISCUSSION

In this study effect of herbal medicinal plant and one Antibiotic on *Salmonella typhimurium* and *Candida albicans* was observed.

Gerard L. Penecilla and Celia P. Magno in 2011 [9] with article “Antibacterial activity of extracts of twelve common medicinal plants from the Philippines” concluded that effect of *Citrus sinensis* is more than *Musa sapientum*. In other research Md. Saidul Islam [10] has been showed *Musa sapientum* have a good antimicrobial effect. This result is different from our result. May be this difference are bacteria type or strain. The use of plant and its products has a long history that began with folk medicine and later has been incorporated into traditional and allopathic medicine [11]. Since antiquity, many plants species have been reported to have pharmacological properties as they are known to posses various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes that are therefore, should be utilized to combat the disease causing pathogens [12-14]. With the advancement in Science and Technology, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs [15].

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