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# Study on antibacterial effect of *Ruta graveolens* extracts on pathogenic bacteria

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**ABSTRACT**

*Regarding to side effects of chemical and synthetic drugs, attention to using herbal products instead of chemical drugs is increased from late years of 20th century. Ruta graveolens is one of the oldest known medical plant which is used in traditional medicine in ancient countries. It is used for different medical purposes and showed a variety of therapeutic effects. Aim of this study is investigating antimicrobial effects of hydro and hydroalcoholic extracts of Ruta graveolens on 10 pathogenic bacteria. Standard strains of Enterococcus faecalis, Staphilococcus aureus, Staphilococcus epidermidis, Strptococcus pneumonia,Streptococcus pyogenesis, Escherichia Coli, Klebsiella pneumonia, Salmonella typhi, seratia marcescens, and psedomonas aerogenesis, are used in this study. Effect of hydro and hydroalcholic extracts of this plant on growth of mentioned bacteria is determined by using disc diffusion method and also by serial macrodilution for measuring MIC in comparison to effects of 11 common antibiotics on the same bacteria. In this study, Hydro and hydroalcoholic extracts of Ruta graveolens did not show inhibitory effect on growth of studied bacteria up to concentration 5mg/ml. It seems that lacking antibacterial effect of the herb extracts on the studied bacteria is due to resistant character of the bacteria and lacking of antibacterial continents in the extracts of the plant.*

**Key words:** *Ruta graveolens*, antibacterial effect, hydroalcholic extract, hydro extract

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## INTRODUCTION

Belief to medical effects of some plants has a long history and was popular in most parts of the old civilizations. It is a part of traditions and customs of nations and different societies [1]. Nowadays most diseases are treated by drugs with chemical origin. Most of them are synthetic, very effective and are developing rapidly. But they have side effects that might be serious sometimes. A positive consideration is growing to substitute herbal drugs for synthetic agents from 2 decades ago. The importance of medical plants is increased in recent years with global attention to using them [2, 3]. World Health Organization (WHO) report indicates that about 80% of world population are using herbal drugs [1,4]. It is more common in some parts of the world especially in old nations such as China, India, American Indians and Iran. It became a new branch in drug industry in some countries. In addition,

about 50% of available and common drugs are originated from plants. Herbal drugs are natural products with homolog continents and are well tolerated agents with no side effects. So they can be used longer time and are more suitable for treating chronic illnesses.

*Ruta graveoloans* is a well known medical plant in ancient civilizations. It is used for treating many diseases such as : Seizure, cough, hypertension, and for wound repair by Asian and European scientist[5]. Antimicrobial effects of extracts of this plant on fungi, protozoa, worms, and bacteria is reported in several studies but the mechanisms are not well known[6].The aim of this study is investigating antibacterial effects on hydro and hydroalcholic extracts of the drug on most common human pathogenic bacteria.

## MATERIAL AND METHODS

Arial organs of *Ruta graveolons* is gathered from high lands of Siakal, northern Iran. Hydro and hydroalcholic (ethanol 70%) extracts are prepared using standard method by rotary extractor(Heidolf 2 G, Germany). One gram of each extract solved in 100ml double distilled water and homogenized by mild heating and stirring, then sterilized by filtration using 0.2 nm syringe filter and stored in 4˚c. Blank discs are soaked with 100 µg, 200 µg, and 300 µg of sterile extract in sterile plates and dried in oven by mild temperature(37˚c for 48 hours). The same procedure is performed for both extracts. Ten Strains are used in this study that are main and common pathogenic bacteria for human : *Staphylococcus aureus* PTCC 1431, *Staphylococcus epidermidis* PTCC1436, *Enterococcus faecalis* PTCC 1237, *Streptococcus pyogenes* PTCC 1447, *Streptococcus pneumonia* PTCC 1240, *Salmonella typhi* PTCC 1609, *Kelebsiella pneumonia* PTCC 1053, *Escherichia coli* PTCC 1554, *Seratia marcescens* PTCC 1609, *pseudomonas aerogenesis* PTCC 1181. They are taken from the center of bacteria and fungi collection, Iranian researches and scientific organization. All strains cultured on TSB and then transferred to TSA. Single colony of each bacteria transferred to Muller Hinton broth to prepare a young well grown culture with Bacterial crowed $10^6$ cfu/ml (using spectrophotometer comparing with 0.5 McFarland solution). This bacterial culture transferred to Muller Hinton Agar to do disc diffusion test that is performed for each strain by using a blank disc as negative control, a standard antibiotic disc as positive control, and 3 test discs containing 100 µg,200 µg, and 300 µg extract. Standard antibiotic discs that are used as positive control and for comparing antimicrobial impact of extracts are ; Penicillin, Oxacillin, Vancomycin, Gentamicin, Tetracycline, Erythromycin, Trimetoprim-sulfametoxazol, Amoxicillin- Clavunic acid, Ampicilin -Sulbactam, and Forazolidin.

The well grown young culture of each strain in Muller Hinton Broth with bacterial crowed $10^6$ cfu/ml is used for serial macrodilution test (after10 fold dilution to take a broth with $10^5$ cfu/ml) . Ten tubes including 5 ml of this broth are used for this test. By adding different amounts of extract to these cultures, serial dilution of extract from 0.5mg/ml to 5mg/ml are prepared and incubated in 35˚c for 18 hours. They are checked for bacterial growth after incubation to measure MIC and cultured on Muller Hinton agar for measuring MBC.

## RESULTS AND DISCUSSION

All studied strains showed resistance to both extracts in disc diffusion test (figure 1 and table1) and in serial dilution test up to 5mg/ml concentration.

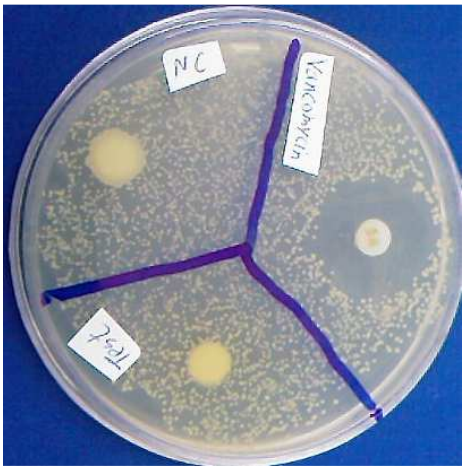


**Figure 1 : Disc diffusion test for *Enterococcus faecalis* with blank disc as negative control,Vancomycin disc as positive control and test disc included 300 µg hydroalcholic extract of *Ruta graveolens*.**

**Table 1 : Antibacterial effect of hydroalcholic extract of *Ruta graveolens* on *Enterococcus faecalis* comparing to antimicrobial agents.**

| Antimicrobial agents | effect |
|---|---|
| Hydroalcholic extract | Resistant |
| Hydro extract | Resistant |
| Trimetoprim-sulfametoxazol | Resistant |
| Oxacillin | Sensitive |
| Vancomycin | Intermediate |
| Penicillin | Resistant |
| Erythromycin | Sensitive |
| Tetracycline | Sensitive |
| Gentamicin | Sensitive |
| Amoxicillin-clavunic acid | Resistant |
| Ampicilin-sulbactam | Sensitive |

Attention to using herbal drugs are increasing in most parts of the world in recent years[7] because they are cheaper and have no side effect. *Ruta graveolens* is a very famous medical plant in Iran from many years ago [8] and several medical effects are introduced for the plant in traditional medicine[9]. Antimicrobial effect of the plant is studied before. Oliva *et al* reported antifungal effect for the plant in two different studies [10,11]. Hydro extract and also 5-metoxipsoralen and 8-metoxipsoralen extracted from this plant showed potent antifungal effect in vitro[10]. In addition, 7-metoxi comarin,7-hydroxi comarin, and 4-hydroxi comarin extracted from this plant showed mild antifungal effect. Antiparasitic effect of *Ruta graveolens* is reported in study of Guarrera and co workers [12] and antibacterial effect of the plant is studied several times. Ivanova found antibacterial effect of the plant on *Staphylococcus aureus*, *Staphylococcus epidermidis*,and *bacillus subtilis* [13]. Study of Olia *et al* showed antibacterial effect of the hydroalcholic extract of the plant on *pseudomonas aeroginosa*[14]. In the study of Alzoreky and Ojala , this plant showed antimicrobial effect on *Staphylococcus aureus*[15,16]. In another study, phenolic component, alkaloids ,and trepnoides extracted from *ruta graveolens* showed antimicrobial effect on *Staphylococcus aureus* and *Bacillus subtilis*[17]. Alzoreky reported that extracts of this plant has more effect on gram positive bacteria than gram negative pathogens[15]In this study we found that both hydro and hydroalcholic (ethanol 70%) extracts of this plant has no antibacterial effect on main human pathogens even those bacteria that reported are sensitive in previous studies. Our founding are compatible with results of study of Saderi *et al* that showed ethanol extract of the plant has no inhibitory effect on *staphylococcus aureus* [18]. The difference might be attributed to the type of extract. Alzoreky used methanol extract (80%methanol with 20% PBS) .In their study MIC was 2.6 mg/ml [15]. In the study of Ojala [16] that used methanol extract(pure methanol) MIC was lower(0.126mg/ml). It seems that there might be more antibacterial components in methanol extract and also in leaves extract and in pure ethanol extract. Saderi *et al* used hydro extract of leaves of the plant on *S.aureus* and found that MIC was 10%v/v [18] that is similar to ethanol (pure) extract of leaves on *P.aeroginosa* [14]. These differences might be due to different resistance of the bacteria. In our study, most used strains showed multidrug resistance to the used antimicrobial agents (results are not shown). We suggest more study in this issue.

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