Lack of antibacterial activity of *Ruta graveolens* extracts against *Enterococcus fecalis*

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Abstract: *Enterococcus fecalis* is responsible for majority of enterococci infections and can cause clinical disorders in adult and pediatrics. In order to adverse effects of synthetic drugs, it has made a positive attitude toward alternative and complementary medicine. *Ruta graveolens* has a wide therapeutic application for various diseases. Aim of this study was to see the effect of this herb on *Enterococcus fecalis* growth. In this investigation we used standard *Enterococcus fecalis*. Effect of hydro-alcoholic, aqueous and methanolic extracts of *Ruta graveolens* on growth of bacteria has been evaluated by disc diffusion and serial dilution method and compared with eight prevalent antibiotics. None of disks with different extracts in the range of 50 to 400µg/µl show any non-growth hallo. Disks with 500µg of all type extracts in comparison with antibiotic disks did not avoid from growth of bacteria. Third test showed the growth of bacteria and ineffectiveness of various amount of extracts. It seems that this ineffectiveness is because of low antibacterial substance against the bacteria in extracts of the herb and high resistant nature of Enterococcus fecalis to antibiotics and it needs more studies.

Keywords: *Ruta*, antimicrobial effect, *Enterococcus fecalis*, hydro-alcoholic, aqueous and methanolic extract.

INTRODUCTION

Enterococcus faecalis is the most common cause of Enterococcus infections (Herdon, 2003). Enterococci are part of the normal flora of the colon and leading cause of nosocomial infections (Andrade *et al.*, 2008). In complicated urinary tract infection (UTI), patients are often treated with antibiotics and will increase the frequency of secondary pathogens such as *Enterococcus faecalis* (Lee *et al.*, 2011). The prevalence of multiple-drug-resistant enterococci has increased around the world (Matsumoto *et al.*, 2011). Previous antimicrobial therapy, urological procedures, the use of urinary catheters, recurrent UTI and hospital stay are theoretically risk factors for developing enterococcal UTI (Yasufuku *et al.*, 2011).

The use of complementary and alternative medicine (CAM) has become increasingly popular worldwide, such that an estimated 38% of American adults reported using a form of CAM in 2006 (Zargari, 1996; Barnes *et al.*, 2008). Rue (*Ruta graveolens*) is the oldest plants used in traditional medicine, especially around the Rasht, in Guilan province, Iran (Zargari, 1996). In Iranian traditional medicine, this plant species is well known and have been used to treat many diseases. Several therapeutic properties such as antibacterial effects have been reported for it (Naghibi-Harat *et al.*, 2009). In this study we aimed to investigate the effect of different types of this plant extracts on the growth of *Enterococcus faecalis in vitro*.

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MATERIALS AND METHODS

Leaves of *Ruta graveolense* were collected in Rasht, Iran, in Aug 2012, and identified in agriculture faculty in Guilan University, Iran. Air-dried leaves were ground in a cross

Table 1: Percentage composition of the oils of aerial parts of *R. graveolens*

SN	Compound	RT	Percentage (%)
1	2-nonanone	10.40	9.1
2	2-nonanol	7.45	1.4
3	geijerene	8.06	1.9
4	geyrene	10.51	11.6
5	2-decanone	11.06	2.3
6	2-heptanol acetate	13.33	18.1
7	cis-isopulegone	14.75	0.6
8	cis-piperitone oxide	13.44	1.4
9	benzene,Trimethyl(1-Methylethyl)	11.25	0.1
10	2-undecanone	14.41	36.2
11	piperitenone	14.70	0.3
12	2-methyl-undecanal	15.45	1.6
13	trans-piperitenone oxide	12.23	0.9
14	2-dodecanone	16.33	1.3
15	trimethyl-8-methylene	14.55	0.4
16	1-dodecanol	17.07	9
17	elemol	17.23	0.9
	Total		97.1%

beater mill equipped with a 1 mm sieve. Hydro-alcholic (ethanol 70%), aqueous and methanolic extracts were prepared using standard method by rotary extractor (Heidolf 2 G, Germany). Different preparations of Ruta extracts were provided. Blank discs were soaked with 50, 100, 150, 200, 250, 300, 350 and 400µg of extract in sterile plates and dried in oven by mild temperature (37°C for 48 hours). The same procedure is performed for both other extracts.

Ruta graveolense hydro-alcoholic dried aerial leaves were (Ethanol/water: 60/40) distilled in a Clevenger-type apparatus for 4 hours. The remained were dried over anhydrous sodium sulfate and kept at 4°C in the sealed brown vials until required. We performed the GC analyses on a HP-6890 gas chromatograph equipped with a split/splitless (20:1) injector (270°C) and a flame ionization detector (270°C). GC-MS analyses were performed on a HP-6890 GC system coupled with a 5973 network mass selective detector and equipped with a HP5-MS capillary fused silica column (30 m × 0.25 mm I.D × 0.32 µm film thickness). The hydro-alcoholic distillation of the aerial parts of *R. graveolens* gave yellowish oil with a yield of 0.4%(v/w), quantitative data were obtained from the integration of the FID peak areas without normalization. In the extract of *R. graveolens* 17 components were identified (Figure 1), which represented about 97.1% of the electronic total detected constituents. The components of the extracts were identified by comparison of their mass retention indices with those reported by previous study and presented in the computer library (table 1). We used *Enterococcus faecalis* PTCC 1237 which was taken from Iranian researches and scientific organization. The strain cultured on TSB and then transferred to TSA. Disc diffusion test was performed for the bacteria by using a blank disc as negative control, a standard antibiotic disc as positive control, and 8 test discs containing 50, 100, 150, 200, 250, 300, 350 and 400µg extracts. Each plate was divided to 4 portions and extracts disks were inserted in it. Then incubated in 35°C for 18 hours; then the size of halo with lack of bacterial growth were measured. Standard antibiotic discs were used: Penicillin, Oxacillin, Vancomycin, Gentamicin, Tetracycline, Erythromycin, Trimetoprim-sulfametoxazol, Amoxicillin- Clavunic acid, Ampicilin-Sulbactam and Forazolidin. We evaluated MIC (Mean Inhibitory Concentration) and then cultured on Muller Hinton agar for measuring MBC (Mean Bactericidal Concentration). For eliminating visual bias, we did linear cultivation on TSA medium for each tube. All of above steps were done for all types of extracts (Aqueous, Hydroalcoholic and methanolic) for two parts of herb (Leaf and stalk) separately. The results were reported observationally.

RESULTS

In the first test, none of the discs in quantities of 50, 100, 150, 200, 250, 300, 350 and 400µg of aqueous, hydro-alcoholic and methanolic extracts inhibited the growth that showed no interdiction effect of extract with the values on the growth of *Enterococcus faecalis*. In the second test, disks containing 500 µg of aqueous, hydro-alcoholic and methanolic extracts compared with antibiotic disks showed no effect on the growth of *Enterococcus faecalis*, whereas the inhibitory effects of antibiotics on bacteria showed growth (Table2). The third test was performed to determine the MIC and MBC in all tubes except control tube. The opacity of the growth of typical bacteria was seen and no effect on the concentrations of 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000 and 9000, and 10000 and 15000 and 20000 mg of hydroalcoholic, methanolic and aqueous extracts were not seen. All tubes were positive in TSA culture medium.

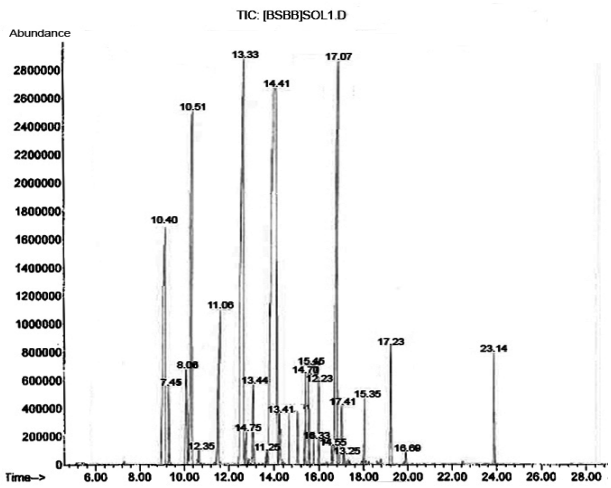

Fig. 1: Composition of the oils of aerial parts of *Ruta graveolens* by gas chromatography

DISCUSSION

Inexpensive and lesser complications of medicinal plants, tends their use in people in most countries (Cowan, 1999). Rue is a native plant of Guilan, in the north of Iran and has always been used in traditional medicine and is noted for its numerous properties (Ahmadi Jalali Moghadam *et al.*, 2012). Studies have shown that this herb is a natural fungicide. Its antifungal properties were investigated in two separate studies by Oliva *et al*. Aqueous extract and 5-methoxy psoralens and 8-methoxy psoralens showed a strong antifungal effect (Oliva *et al.*, 1999). The 7-methoxy-coumarin, 7-hydroxy-coumarin and 4-hydroxy-coumarin extracted from this plant showed moderate antifungal activity (Oliva *et al.*, 2003). Its antibacterial effect has been examined in several studies. Ivanova *et al*. demonstrated its antibacterial effect on *Staphylococcus aureus* and *Staphylococcus epidermidis* and *Bacillus subtilis* (Ivanova *et al.*, 2005). Olia et al showed its antibacterial effect on *Pseudomonas aeruginosa* (Olia *et al.*, 2013).

Ojala studies have been shown antibacterial effect of Rue on *S. aureus* (Ojala *et al.*, 2000). Phenolic, alkaloids and terpenoids compounds in *R. graveolense* have shown

Table 2: Comparison of extracts of herb on the growth of *Enterococcus faecalis* with standard antibiotic disks

Content of disk	Size of lack of growth halo	Results
Hydro alcoholic extract	0	Resistance
Methanolic extract	0	Resistance
Aqueous extract	0	Resistance
Cotrimoxazole	18	Sensitive
Oxacililin	12	Intermediate
Vancomycin	22	Sensitive
Erythromycin	27	Sensitive
Furazolidone	23	Sensitive
Tetracycline	19	Sensitive
Amoxicillin - Clavunic acid	2	Resistance
Ampicillin - Sulbactam	20	Sensitive

antibacterial effect on *Staphylococcus aureus* and *Bacillus subtilis* (Saderi *et al*., 2006; Al-Bakri and Afifi, 2007). The study showed that the extract of this herb affect gram-positive bacteria more than gram negative (Alzoreky and Nakahara, 2003). In our study, even high concentrations of hydro-alcoholic, aqueous and methanolic extracts (20mg/ml) did not show inhibitory effect on *Enterococcus faecalis in vitro*. Saderi et al showed that its hydro-alcoholic extract had no inhibitory effects on the growth of *S. aureus* (Saderi *et al.*, 2006). In Ojala studies methanolic extract of leaf of *R. graveolense* showed antibacterial effect on Staphylococcus aureus, which can because of the presence of antimicrobial substances in their leaves than stems, and extraction by methanol (Ojala *et al.*, 2000). In our study, even with 40% volume concentration of 20mg/ml showed no effect in three different types of extracts. In Ojala study methanolic extract with agar dilution method revealed antibacterial effect in MIC of 0.126mg/ml (Ojala *et al.*, 2000) and it was a very different MIC for *S. aureus* obtained in study of Alzoreky (Alzoreky and Nakahara, 2000). In Saderi *et al*. study, aqueous extract of dried seeds and stems showed the least impact and the MIC for *Staphylococcus aureus* was obtained 10% of concentration in volume (Saderi *et al.*, 2006). In Olia *et al*. study ethanolic extract its leaves showed antibacterial effect on *P. aeruginosa* (Olia *et al.*, 2013). Although our results showed that Rue of our region have enough active constitutes, due to the differences in our results with some other studies, it seems that *Enterococcus faecalis* is more resistant to different extracts from *R. graveolense* (Aqueous, Hydro-alcoholic and methanolic), but this point needs further studies.

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