

## INVESTIGATING THE ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF THE MEDICINAL PLANT *RUTA GRAVEOLENS*

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### ABSTRACT

*Ruta graveolens* is a commonly used curative plant and indigenous to the Mediterranean region. In the present study, the ethanolic extraction of *R. graveolens* leaves and stems was tested for antibacterial effects against *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*. The leaves (60g) and stems (60g) of *R. graveolens* were collected; shade dried and powdered using a grinder. After soaking the powder in 200 ml of 96% ethanol for 24 hours and filtering it, the precipitant was air dried and 100 ml of distilled water was added to it. Both leaves and stems extracts showed inhibitory properties on gram negative and gram positive bacteria. *S. pyogenes* and *S. aureus* were strongly affected by the stems extract while *E.coli* had the highest inhibition zones among the bacteria which were treated with the leaves extract. The aim of the present study is to estimate the antibacterial efficacy of *R. graveolens* against bacterial isolates and that could open the possibility for the development of novel antibiotics.

**KEYWORDS** : *Ruta graveolens*, Antimicrobial, Antibacterial, Ethanol extract, Pathogenic bacteria, Medicinal herb

### 1. INTRODUCTION

Herbal medicines have been commonly used around the world for centuries, especially in developing countries where they combine it with synthetic drugs. *Ruta graveolens* is one of the widely used medicinal herbs; commonly known as “Rue” or “Sudab” in some countries of Asia and belongs to the family Rutaceae. The shrub-like evergreen plant is native to southeastern Europe. It has strong smelling and a bitter flavor. The stem is woody at the base and foliage is bluish-green, 2-3 pinnate and alternate (Ríos, 2015; Toby et al., 2011).

The plant has been known as pain reliever and curing dermatitis, eczema, inflammation, rheumatic disease, fibromyalgia, ulcers, and acts as abortion-inducing herb, insectifuge and antitoxin (Fadlalla et al., 2011; Ríos, 2015; Woo et al., 2012). Moreover, the volatile oils of *R. graveolens* showed curative treatment for gastrointestinal cramps after being mixed with water and sugar and then taken (Ravindran et al., 2012). However, therapeutic dosages of *R.*

*graveolens* cause sleep disturbance, depression, vertigo, muscular spasm and weakness. In addition, it could lead to photoirritation, nephrotoxicity and hepatotoxicity (Aronson, 2016).

Previous studies showed that the plant was noticed to reduce spermatogenesis in Wistar rats. Rahim et al. (2009) demonstrated that *R. graveolens* ethanolic extract decreases the level of testosterone hormone and motility of sperms. Sailani & Moeini (2007) suggested that the extract could have an effect on the pituitary gland and lead to a decrease in androgen production which is responsible for sperm production. The effect is not only limited to male rats but it was found that chloroform extract of *R. graveolens* significantly reduces fertility in female rats (Kong et al., 1989). Additionally, Nasirinezhad et al. (2016) stated that the alcohol extract of *R. graveolens* does not affect the levels of follicle stimulating hormone (FSH) and luteal hormone (LH) and could help in birth control. The mildly toxic plant has shown therapeutic action, however, caution is needed especially in case of long term

consumption, besides that self- treatment is not recommended (Freire et al., 2010).

The phytochemical screening of *R. graveolens* documented that the plant has primary and secondary metabolites. The secondary metabolites mainly include bioactive compounds such as phenolics, flavonoids, alkaloids, terpenoids, glycosides, diterpenes, triterpenes . The secondary metabolites have been known to have defense mechanisms against a wide range of pathogens and pests (Amabye & Shalkh, 2015). *R. graveolens* has been also found to protect DNA strands from breakage and potential mutations, besides its inhibitory action against the proliferation of cancer cells (Fadlalla et al., 2011; Ríos, 2015). Researchers have demonstrated the action of the *R. graveolens* essential oils and extracts as significant antimicrobial agents. They reported the antibacterial action against a variety of bacterial pathogens; the examined parts of the plants were either aerial parts together or leaves.

Fewer studies have investigated the action of stems. Pandey et al. (2011) were the first to document the inhibitory action of the *R. graveolens* stem against bacteria and fungi. They found that ethanol, methanol, chloroform and aqueous extracts of the stem have significant antibacterial and antifungal action. In contradiction to Ahmadi et al. (2012), where they did not observe any antibacterial effect of aqueous and ethanol 70% extract on pathogenic bacteria. They attributed their results either to the type of the used extract or the bacterial strain that showed resistance to the tested antibiotics.

On the other hand, Reddy et al. (2016) stated the importance of *R. graveolens* essential oils, where they examined the action of oils of aerial parts on gram negative and gram positive bacteria and also against fungi and yeast.

The aim of this study is to determine the antibacterial action of the ethanol extracts of leaves and stems against gram negative and gram positive bacterial strains.

## 2. MATERIALS AND METHOD

### - Bacterial specimens:

The bacterial samples (*P. aeruginosa*, *E.coli*, *S. aureus*, *E. faecalis*, and *S. pyogenes*) were collected from Azadi hospital and cultured on nutrient, blood, and MacConkey agar. The study was conducted in the research laboratory of the Duhok Technical Institute, Duhok Polytechnic University.

### -Collection of *Ruta graveolens* and preparation of the ethanol extract:

The medicinal plant *R. graveolens* was obtained from a local plant nursery in Duhok governorate, Kurdistan region of Iraq and identified in the research laboratory of the Duhok Technical Institute. The (60g) of stems and (60g) of leaves were separated; shade dried and powdered using a grinder. Each powder was soaked in 200 ml of 96% ethanol for 24 hours and filtered through layers of gauze. The filtrate was subsequently left to dry at room temperature. The stock solution was prepared by adding 100ml of distilled water to the dried filtrate and the concentration 20, 40, 60, 80 and 100 mg/ml were used in the study (Harish et al., 2014).

### -Well diffusion method:

According to Balouiri et al. (2016), the agar diffusion method was performed by using Mueller Hinton agar, the recommended growth medium for antimicrobial susceptibility tests. A number of wells were made on each agar plate using sterile glass tubes. After the bacterial isolates have been swabbed uniformly on plates, the wells were filled with drops of each concentration of the ethanol extract besides adding tetracycline 10mg discs as positive control and distilled water as negative control. Finally the plates were left for 15 minutes at room temperature and then incubated at 37°C for 24hrs and the inhibition zones were measured in mm.

## RESULTS AND DISCUSSION

Different communities have been using the medicinal herbs for centuries as traditional natural remedies. Approximately 80% of the global population commonly depends on the medicinal plants as they are inexpensive and easy to obtain and they may use them along with synthetic medicines, especially in developing regions (Mbuni et al., 2020). The phytochemical constituents of these plants proved their therapeutic efficacy against wide range of pathogens (Škrovánková et al., 2012).

*R. graveolens* has been known to be one of the important herbal medicines which is considered a rich source of natural bioactive substances including alkaloids, volatile oils, coumarins, and phenolic acids and flavonoids. The extracts (alcohol and aqueous) and volatile oils of *R. graveolens* have shown antimicrobial,

anthelmintic and antiparasitic properties besides acting as antiphlogistic, antipyretic, anti-diarrheal, anti-rheumatic, analgesic (Orlanda & Nascimento, 2015; Malik et al.,2017).

The findings of the present study demonstrated the antibacterial effect of *R. graveolens* extract on gram negative and gram positive bacteria. The ethanol extract of leaves showed a very close effect on all tested bacteria (Figure1). *E.coli* was the most sensitive bacteria among all the strains to the leaves extract with maximum inhibition zone (15.3 mm) at concentration of 100 mg/ml. However, the concentration of 20 mg/ml did not show any inhibition. This result comes in agreement with Amabye & Shalkh (2015) where they found that

the ethanolic extraction of *R. graveolens* leaves has a significant effect on *E.coli*. *S. pyogenes* reported high inhibition zone (9 mm) at 100 mg/ml. Similar to *E.coli*, the 20 mg/ml concentration exhibited a weak effect with inhibition zone of (1 mm) (Table 1). These finding may indicate that the effect of ethanol decreases obviously as the dilution increase. In comparison to methanol and chloroform extract that are more effective against bacterial isolates at higher dilutions (Amabye & Shalkh, 2015). *S. aureus*, *E. faecalis* and *P. aeruginosa*, experienced relatively the same action of leaves extract with maximum inhibition zones at 100 mg/ml.

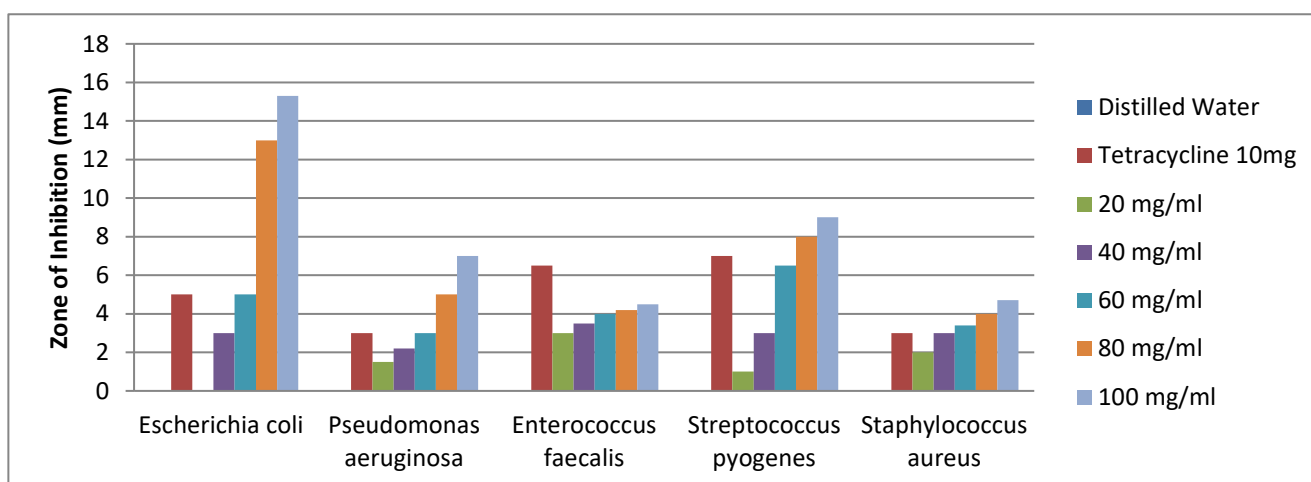


Fig.( 1):- The effects of ethanol extract of leaves on the growth of bacterial strains.

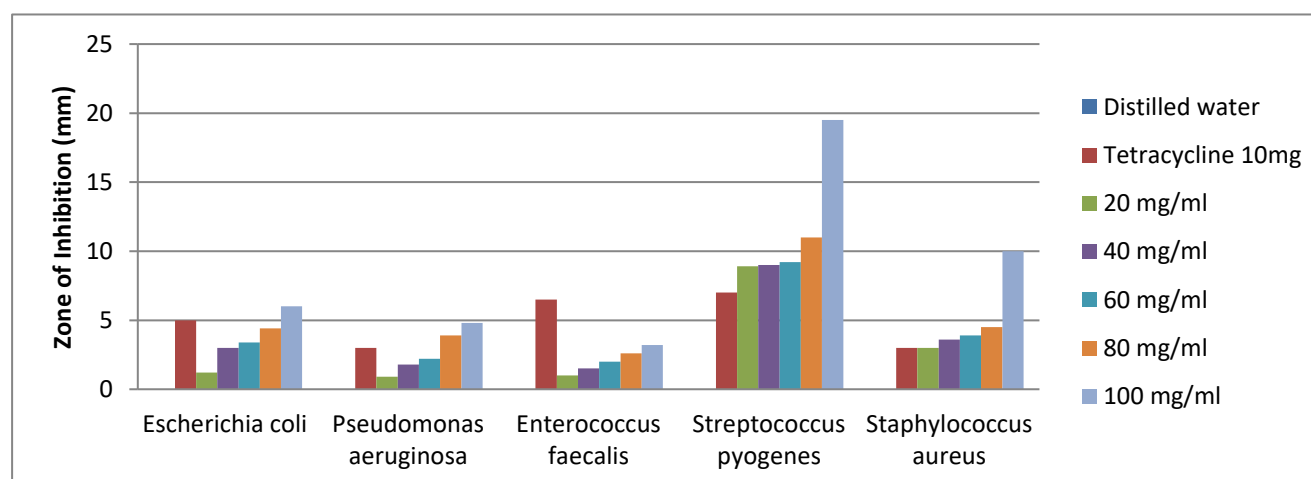


Fig.( 2):- The effects of ethanol extract of stems on the growth of bacterial strains.

**Table( 1):-** Descriptive statistics of the effects of the leaves extract on all bacteria

Descriptive statistics	<i>S. pyogenes</i>	<i>S. aureus</i>	<i>E. faecalis</i>	<i>E.coli</i>	<i>P.aeruginosa</i>
Mean	5.5	3.42	3.84	7.26	3.74
Standard Deviation	3.391165	1.020784	0.594138	6.586957	2.244549
Standard Error	1.516575	0.456509	0.265707	2.945777	1.003793
Lower 95% CL Mean	1.289312	2.152529	3.10228	-0.91879	0.953024
Upper 95% CL Mean	9.710688	4.687471	4.57772	15.43879	6.526976
Minimum	1	2	3	0	1.5
Maximum	9	4.7	4.5	15.3	7

**Table( 2):-** Descriptive statistics of the effects of the stems extract on all bacteria.

Descriptive statistics	<i>S.pyogene s</i>	<i>S. aureus</i>	<i>E. faecalis</i>	<i>E.coli</i>	<i>P.aeruginosa</i>
Mean	11.52	5	2.06	3.6	2.72
Standard Deviation	4.542797	2.846928	0.870632	1.772004	1.592796
Standard Error	2.0316	1.273185	0.389359	0.792465	0.71232
Lower 95% CL Mean	5.879373	1.465072	0.978968	1.399766	0.742282
Upper 95% CL Mean	17.16063	8.534928	3.141032	5.800234	4.697718
Minimum	8.9	3	1	1.2	0.9
Maximum	19.5	10	3.2	6	4.8

**Table( 3):-**ANOVA test compares the efficacy of leaves and stem extracts on all bacteria.

Power		(α=0.05)				
Between	1	0.6498	0.6498	0.0427	0.83711	0.05472
Within (Error)	48	729.9424	15.20713			
Adjusted Total	49	730.5922				
Total	50					

**Table( 4):-** ANOVA test compares the efficacy of leaves and stem extracts on *S. pyogenes* and *S. aureus* , showing the significant effect of stems extract on gram positive bacteria.

Power		(α=0.05)				
Between	1	72.2	72.2	4.6048	0.04577	0.52827
Within (Error)	18	282.228	15.67933			
Adjusted Total	19	354.428				
Total	20					

\*The NCSS statistical software was used for data analysis.

\*Sum of squares (SS), Mean square (MS).

The gram negative bacteria showed less sensitivity to the plant extract in contrast to gram positive bacteria, especially against the stems extract. The resistance mechanism in gram

negative bacteria is attributed to their outer membrane which acts as strong barrier. On the contrary, the membrane of gram positive bacteria is more permeable to the diffusion of

the molecules of antibiotics (Atef et al., 2019). According to previous studies, the volatile oils of the aired parts of the *R. graveolens* reported high antibacterial activity against gram positive bacteria (Orlanda & Nascimento, 2015; Reddy & Al-Rajab, 2016). In addition, this study showed the strong action of stems extract against gram positive bacteria. This could indicate that stems extract has the greatest contribution on the inhibition of gram positive strains rather than leaves (Table 4). On the other hand, the findings of Pushpa et al. (2015) disagree with the previous conclusion, where they found that the methanolic extract of the aerial parts of *R. graveolens* has stronger inhibitory action on gram negative bacteria. The strain of bacteria and the type of the solvent that is used for extraction could lead to this contradiction in results (Pushpa et al., 2015). In this study tetracycline 10mg was used as positive control on all bacterial strains. *E. faecalis* and *S. pyogenes* were the most sensitive bacteria with inhibition zones (6.5 mm and 7mm) respectively. *P. aeruginosa* and *S. aureus* were less affected by tetracycline.

The stems extract exhibited a relatively strong action against *S. pyogenes* and *S. aureus*. They were the most sensitive bacteria to the extract (Figure 2). The highest inhibition zones (8.9 mm at 20 mg/ml, 19.5 mm at 100 mg/ml) were observed in *S. pyogenes*. However, *E. faecalis*, did show resistance to the stems and leaves extracts (3.2 mm at 100 mg/ml and 4.5mm at 100 mg/ml) respectively. The resistance of *E. faecalis* to aqueous, ethanol 70%, and methanol extract of *R. graveolens* was also observed by Saeidinia et al. (2016). *P. aeruginosa* and *E.coli* were comparatively more resistant to the stem extract. Pandey et al. (2011) documented that the ethanol extract of stems showed a powerful action against *S. aureus* and *Bacillus subtilis* while *P. aeruginosa* showed high resistance. Considering these findings and the data of this study it can be inferred that *R. graveolens* stems extract has demonstrated its efficacy against gram positive bacteria. The extract of the stem contains alkaloidal, phenolics, tannins, flavonoids and the alkaloids are responsible for the obvious antimicrobial activity against pathogens.

## CONCLUSION

This study showed the antibacterial efficiency of *R. graveolens* extracts against different bacterial strains. These findings and in accord to the previous studies demonstrate the importance of *R. graveolens* as natural antibacterial and prospects for the contribution in pharmaceutical industry.

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