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ANTIBACTERIAL ACTIVITY OF HEXANIC EXTRACT OF *RUTA GRAVEOLENS* ON *KLEBSIELLA PNEUMONIAE*- A COMMON POST- ORTHOPEDIC SURGERY INFECTION

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ABSTRACT: Introduction: *Klebsiella pneumoniae* is one of the most frequent infection organism following orthopedic surgeries and specifically total hip arthroplasty. Recently, attitude toward herbal medicine is being popular both in people and health care systems. *Ruta graveolens* is a traditional herbal remedy Iranian medicine and some other countries with a wide spectrum of therapeutic utilities. Our purpose was to determine the effects of this medicinal plant on *Klebsiella pneumoniae* growth. **Materials and methods:** Standard *Klebsiella pneumoniae* was used. We assessed the hexane extracts of both leaf and stark of *Ruta graveolens* on growth of bacterial disc diffusion and serial dilution methods. We compared the results with some standard frequent chemical antibiotics. **Results:** Our results showed that all doses of leave extract including 200, 250, 300, 350 and 400 micrograms of stark extract had inhibited the growth. In disc diffusion test 500 µg of hexanic extract prevent the growth of bacteria rather than antibiotic discs. We determined minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) by doing their methods. **Conclusion:** Hexanic extract of Ruta can affect the growth of *Klebsiella pneumoniae* both for bactericidal and inhibitory effects. It can be a good candidate for more research *in vivo* to introduce new agent for treating this type of infection.

INTRODUCTION: Total Joint Arthroplasty (TJA) is a safe, important and cost-benefit surgery which can progress the quality of life and survive the activity of patients who have destructed hip or knee joints [1]. Despite of very successful outcomes of TJA in different cases, complications related to procedure are not unavoidable.

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One of them is Periprosthetic Joint Infection (PJI) which is now a major mechanism of implant failure lead to revision arthroplasty [2].

There are many bacteria can make prosthesis-related infections, including *Propionibacterium acnes*, *Staphylococcus aureus* (*S. aureus*) (both methicillin-resistant *S. aureus* (MRSA) and coagulase-negative *Staphylococci* like *Staphylo-coccus epidermidis*, *Staphylo-coccus hemolyticus* and *etc*.), *P. aeruginosa*, *Providencia*, *Enterococci*, *Haemophilus influenzae*, *Streptococcus viridans*, Citrobacter, *Escherichia coli*, *Lactobacillus*, *Klebsiella pneumoniae*, *Corynebacterium* and so on[3].

Klebsiella is one of the most organisms responsible for PJI [4]. It is an anaerobic, rod, gram-negative bacterium. It is covered by a poly-saccharide capsule which can lead to big shape in gram staining and make resistance against various host defence mechanisms. This bacterium is account for respiratory tract infection like primary pneumonia, pharynx and nasal mucosa infections [4]. Since very ancient times, people have used plants to treat infectious diseases and investigations showed that plant material have proven remedial effects over time. Nowadays, many countries use plants to treat different maladies including infectious diseases of the respiratory, gastro-intestinal, urinary and biliary systems [5].

Ruta gravelones is one of traditional medicine remedies for different diseases. It is used for a long time for treatment of anti-seizure, wound healer, anti-cough and decreased blood pressure and has anti-fungal, anti-parasitic worms, reducing some nervous system functions, contraception and fetal abortion [6]. We previously worked on its anti-seizure [7, 8] and antibacterial effect on another bacterium [9]. Although there were many clinical and laboratory studies, several mechanisms and potential effects of this plant and its composition of compounds is not known [10]. We evaluated the efficacy of hexanic extract of *Ruta graveolense* on the growth of *Klebsiella pneumoniae in-vitro*.

MATERIALS AND METHODS: We collected the leaves part of plant in Rasht, Northern Iran in May 2015 and validated it by an agriculture expert in medicinal plant in Guilan University. Leaves were dried by air on the ground and was fragmented to little pieces of 1 millimeter by mixer. The hexanic extract was prepared by using standard method with rotary evaporator (Heidolf 2 G, Germany). Several doses of Ruta extracts were prepared. We steeped blank discs with doses ranged between 50 to 400 µg of herbal extract in sterile plates, then dried them by mild temperature in oven (37 °C for 48 hours). We performed all analyses of compositions of the herb based on previous work. Preparation of herbal extract is described before [9]. We used *Klebsiella pneumoniae* which was taken from Iranian researches and scientific organization. Culture of the strain was done on TSB (Tryptic Soy Broth, Difco and BBL, NJ USA) medium then TSA (Tryptic Soy Agar, Difco and BBL, NJ USA). Disc diffusion test were used on the bacteria with a blank disc as negative control and a standard antibiotic disc for positive control.

We also used eight test discs with different doses of extracts. We divided every plate to 4 parts and inserted the extracts discs in it. These discs were incubated in 35 °C for 20 hours and thereafter the size of halo that had no bacterial growth were measured and recorded.

We utilized eight standard antibiotic discs: Amoxicillin- clavunic acid, forazolidin, penicillin, gentamicin, tetracycline, ampicilin-sulbactam, oxacillin, erythromycin, trimetoprim-sulfametoxazol and vancomycin. Mean inhibitory concentration (MIC) was performed for all of the discs. Then it was cultured on Mueller-Hinton agar culture medium (Merck, Germany) for measuring mean bactericidal concentration (MBC). In order to removing visual bias, linear cultivation was done for each tube. All of mentioned processes were done for two different parts of Ruta (both leaf and stark) separately.

RESULTS: In the first investigation step, no discs of 50, 100, 150 micrograms of hexane, stark extracts inhibited the growth which showed no interdiction effect of extract with the values on the growth of *Klebsiella pneumoniae* but all doses of leave extract and doses of 200, 250, 300, 350 and 400 micrograms of stark extract had inhibited the growth.

In the second investigation step, discs with 500 micrograms of hexane stark and leave extracts compared with cotrimoxazole antibiotic discs, oxacililin, vancomycin, erythromycin, furazolidone, tetracycline, amoxicillin - clavunic acid and ampicillin - sulbactam showed significant effect on *Klebsiella pneumonia* growth (**Table 1**).

The aim of third investigation step was to assess the MIC and MBC in all tubes except control one. The opacity of the growth of typical bacteria was seen and effect on the concentrations of 3000 to 20000 mg of hexanic extract (stark and leave separately) were evaluated. All tubes were positive in TSA culture medium except doses more than 9000 (leave extract) and more than15000 (stark extract).

TABLE 1: COMPARISON OF EXTRACT OF HERB ON THE GROWTH OF *KLEBSIELLA PNEUMONIAE* WITH STANDARD ANTIBIOTIC DISCS

Content of disc	Size of lack of growth halo	Interpretation of results
Hexanic stark extract	23	Sensitive
Hexanic leaves extract	27	Sensitive
Cotrimoxazole	16	Sensitive
Oxacililin	13	Intermediate
Vancomycin	24	Sensitive
Erythromycin	5	Resistance
Furazolidone	20	Sensitive
Tetracycline	11	Intermediate
Amoxicillin - Clavunic acid	4	Resistance
Ampicillin - Sulbactam	14	Sensitive

DISCUSSION: Secondary compounds of traditional herbs have a high potential for research in introducing new kinds of bactericidal agents. These metabolites are including phenolics, alkaloids, terpenoids, steroids, saponins, essential oil and others. Many various activities are attributed to these kind of metabolites [11].

Naturally occurring botanical compounds contain a broad range of chemical active ingredients that can interfere with the biological processes of microbes and thus interrupt its life cycle and dispersal and reduce harm to humans and animals. In this study we evaluated the hexanic extract of Ruta. We previously examined the effects of hydro-alcoholic, aqueous and methanolic extracts of this herb with no effect on *Klebsiella pneumoniae* and other pathogens [12 - 14]. Here, we showed that stark and leaves hexanic extracts of this herb have a powerful inhibitory and bactericidal effects on *Kelbsiella pneumoniae*. Leaves extract had more potent efficacy rather than stark extract. Due to our knowledge this is the first study of hexanic extract of *Ruta graveloens* on *Klebsiella pneumoniae*.

Ivanova and colleagues showed that all the extracts (MeOH, petroleum ether and EtOAc and H_2O/ MeOH extracts) showed no activity against the Gram (-) strain Escherichia coli and the fungus Candida albicans. However, they showed the inhibitory effects and a clear selectivity for the studied Gram positive microorganisms [15]. It is reported in the literature the moderate antifungal results of the 7-methoxy-coumarin, 7-hydroxy-coumarin and 4-hydroxy-coumarin which were extracted [16]. Antibacterial activity of Ruta is reported in several studies [17, 18]. In an *in vitro* investigation, the antibacterial effect of the herb was exhibited on *Pseudomonas aeroginosa* [19].

In two other studies, antibacterial effect of Ruta on *Staphylococcus aureus* was reported [17, 18]. Al-Bakri and Afifi expressed that some trepenoids, alkaloids and phenolic constitutes of *Ruta graveolense* are accounted for its antibacterial activity on *Bacillus subtilis* and *Staphylococcus aureus* [20]. Chacko *et al*., demonstrated that in evaluation of three different solvents included hexane, petroleum ether and methanol the best of them in larvicidal activity was petroleum ether solvent for *R. graveolens* [21].

In our study, hexanic extracts showed inhibitory effect on *Klebsiella pneumoniae in-vitro*. Although we showed the inhibitory effect of the herb, it was stronger than some of common antibiotics for inhibition. Saderi and *et al*., demonstrated that hydro alcoholic extract extracted with ethanol cannot inhibit *S. aureus* growth [22]. Chunha *et al*., showed that analgesic effect of hexanic extract of *Ruta gravelones* was higher than its other extracts [23]. Based on previous studies the better effects of hexanic extract of Rue in our study is due to presence of a large amount of substances such as flavonoids (rutin), alkaloids (quunolines, furano-coumarin and pyranocoumarin), coumarins and essential oils (2-nananone and 2-pyranocoumarin) [9, 23]. The better effects of leaves extract resulted from high level of active constitutes in it rather than stark.

CONCLUSION: *Klebsiella pneumoniae* is a critical cause of PJI after orthopedic surgeries and finding a natural product for prophylaxis may be helpful in reducing the rate of PJI. Due to our results, we concluded that both hexanic stark and leaves extracts of *R. graveolens* had inhibitory and bactericidal effects on *Klebsiella pneumoniae* and more researches are needed *in-vivo* to use this herb as a natural antibiotic drug.

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CONFLICT OF INTEREST: All authors declare any conflict of interest.

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