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Original Article

Evaluation of Antibacterial Activity of Aqueous and Ethanol Extracts of *Rumex Alveollatus* Against *Klebsiella Pneumoniae*

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ABSTRACT

Introduction: Antimicrobial resistance in *Klebsiella pneumoniae* has been on the increase over the years. Therefore, numerous endeavors have been carried out so as to discover another active plant combination as a suitable substitute for antibiotics. This is an experiment of the activities of anti-bacteria from the extract from *Rumexa alveoli* against *ebsiella pneumonia*.

Materials and Methods: This study was performed on the standard strain of *Klebsiella pneumonia* and several clinical strains. The aqueous and ethanol extracts of *Rumex alveollatus;* formulated by the Soxhlet apparatus. The antibacterial activities of the extracts were analyzed with the use of the disk-diffusion method. Furthermore, the minimal-inhibitory-concentration (MIC) and the MBC were examined by the micro-dilution method. The database was evaluated by the SPSS software.

Findings: Inhibitory zone of ethanol (500 mg/mL) on the standard and clinical strains of *Klebsiella pneumoniae* were 18, 17, 17, 15, 16, and 16 mm, respectively, and also an aqueous extract did not have an antimicrobial effect. MIC and MBC results for ethanol extracts were 125 and 250 mg/mL, respectively.

Conclusion: The antibacterial activities of the *Rumex alveollatus* ethanol and aqueous extract tested against bacteria were good. Thus, the findings of this research can be a beneficial report concerning their active role in the infection control.

Keywords: Ethanolic Extracts, Aqueous Extracts, Antibacterial Activity, *Rumex Alveollatus*.

1. Introduction

Klebsiella pneumoniae is a gram-negative coccobacillus with a thick capsule that colonizes the respiratory and gastrointestinal tract, but is also a recognized opportunistic pathogen leading to various infections including nasopharyngeal, osteomyelitis, gastrointestinal, skin, biliary, urinary tract infections, and bacteremia [1,2]. The improper and continuous use of these substances has led to the phenomenon of antibiotic resistance and the emergence of resistant strains and treatment due to *Klebsiella pneumoniae*. The resistance phenomenon to antibiotics among pathogenic bacteria is a topic that is considered a problem worldwide today [2,3]. The use of antibacterial properties of plants has introduced a new approach to antibiotic resistance and replacement. In the distant past, much attention has been paid to plant extracts and compounds with different biological properties. Achieving new applications as adjunctive medicines, realizing the health values of plants, and finally discovering the effective substances with antimicrobial and antitumor properties of plants have helped in the development of herbal medicine [4,5]. Herbal antimicrobial compounds are not only effective in treating infectious diseases, but also simultaneously lack some of the side effects of other antimicrobial compounds. Plants have an unlimited ability to synthesize aromatic compounds, most of which are phenolic compounds or their derivatives. These compounds are the secondary metabolites of plants and have significant therapeutic effects against viruses, bacteria, and fungi [5,6]. Today, various species of plants have been proposed as plant extracts or essential oils for the treatment of bacterial infections. *Rumex Alveollatus* is one of the plants that have been evaluated in different parts of traditional medicine. *Rumex Alveollatus* grows in the mountainous regions of western Iran at an altitude of 1400-1200 meters. This plant has the straight and long roots. The leaves are fleshy and the flowers are green and grow on the cluster and in the upper part of the leaves. *Rumex Alveollatus* is used in the traditional medicine to treat tumors, liver disease, constipation, heart problems, spleen disease, hiccups, bloating, asthma, bronchitis, indigestion, scabies, toothache, and nausea [7-9]. This plant's composition contains many biologically active substances such as flavonoids and anthraquinones. Especially in the root of this plant, carotenoids, vitamins (especially vitamin C), proteins, lipids, and organic acids have been identified. The antimicrobial properties of aqueous and ethanolic extracts of different plant species have been reported on a wide range of bacteria from different geographical areas [10,11]. Because of the existence of numerous phytochemicals having substantial antibacterial potential in *Rumex Alveollatus* on one side of the coin and the universal issue of drug resistance in bacteria, on the other side of the coin, it is of great necessity to execute laboratory researches for defining the quality and scope of the effect of the mentioned materials. The pathogen is done. Therefore, this study aimed to determine the antibacterial effect of aqueous and ethanolic extract of *Rumex Alveollatus* on a number of standard and clinical strains of *Klebsiella pneumoniae*.

2. Materials and Methods

2.1. Identification and plant collection

This experimental study was performed with ethics code IR.KAUMS.REC.1399.020. This experimental study was conducted from June to November 2021. In May, the leaves and branches of the *Rumex Alveollatus* plant were collected and used

after approval by the ANRR Center of the district. The plant was dried in the shade at 25-30 °C, and then it was powdered.

2.2. Preparation of ethanolic and aqueous extracts

To extract ethanolic and aqueous extracts by the Soxhlet method, it was administered by 50 g of *Rumex Alveollatus* powder. The extraction process was conducted separately by using 250 mL of 100% water as the solvent and ethanol. Subsequently, 8 hours later, the obtained alcoholic extracts were positioned in the open air at the temperature of 25-27 °C to evaporate the excess alcohol and obtain a pure extract. Then, the extract was stored in turbid vials at 4 °C [12].

2.3. Preparation of the standard strains

The tested microorganisms including standard strains of *Klebsiella pneumonia* (ATCC:10031) were prepared as lyophilized from the collection of microorganisms of the Scientific and Industrial Research Organization of Iran. Also in this study, 5 clinical isolates of *Klebsiella pneumoniae* were used. Isolates were cultured on McConkey agar and blood agar medium, and then specific diagnostic tests were performed to identify *Klebsiella pneumonia*. To prepare the microbial suspension from fresh and young culture, several colonies of bacteria were transferred to Müller Hinton Broth (Himedia, India) culturing medium to achieve the turbidity of the microbial suspension that was prepared according to 0.5 Mac Farland tube (concentration equivalent to 1.5 × 108 bacteria per milliliter) [13]. Given that the samples are taken from the laboratory and identified, there is no ethical consideration.

2.4. Preparation of different dilutions of *Rumex Alveollatus* extraction

To examine the antimicrobial impacts of ethanolic and aqueous extracts, 2 g of ethanolic and aqueous extract was put in to a tube comprising of 4 mL of MHB culture medium embodying 20% of DMSO and shaken well to be completely dissolve. Thus, the extract concentration was 500 mg/mL. Then concentrations of 500, 250, 125, 62.5, 31.2, 5, and 15.6 mg/mL of the extract were prepared. In this study, the antimicrobial effect of ethanolic and an aqueous extract was investigated by both disk diffusion and micro-dilution methods [14].

2.5. Evaluation of antibacterial activity of extracts by disk diffusion method

Blank disks were placed in different concentrations of the prepared extract for an hour to absorb the extract. The discs were then stored in a dry, the environment was steriled for sterilization in sterile vials. Thereafter, by using the disk diffusion technique, a microbial suspension of 100 µL with a concentration of 1.5 × 106 f cfu/mL was dissipated equally on the exterior of the MH agar medium. Thus, from different concentrations, the prepared disk was placed on the plate surface at a distance of 2.5 cm. The adverse influence was the DMSO solution experiment with the imipenem antibiotic as a favorable control. Every inoculated medium were exposed to 37 °C for 24 hours. After a certain time, the microbial cultures were examined for the formation or non-formation of the growth inhibition zone around the discs, and the growth inhibition zone diameter was measured in millimeters. Each series of experiments was repeated 3 times for each extract and each of the studied bacteria and the standard of the growth inhibition zone diameter was calculated [13]. Likewise, the assessment of the minimum lethal concentration (MBC) and the minimum inhibitory concentration (MIC) of the extract was carried out.

After the subsequent preparation of different concentrations of the extract, MBC and MIC values were defined by the microtiter plate method. For this, 100 µL of every concentration was put into the wells starting from columns one to ten of 96 sterile well microplates (every column approximates to 1 concentration, respectively). Then, the microbial suspension of 100 µL with a concentration of 1.56 × 106 cfu/mL was added to each well. Column 11 was inserted as a negative influence 200 µL of MHB medium. The microbial suspension of 100 µL and 100 µL of MHB medium were placed into column 12 which served as the positive control. When inoculation was done, the light absorption by the walls was examined by using the ELISA reader at a wavelength of approximately 450 NM. The microplate was then incubated at 37 °C. After 24 hours, the light absorption by the walls was examined again by an ELISA reader at approximately 450 nm. Each test was done 3 times and average of the results was measured. The last well that was transparent, which means the minimal concentration of the extract that inhibited the bacteria development, was considered MIC [13]. To determine the minimum lethal concentration, 20 µL of the extract from the MIC well and four transparent pre-MIC wells were cultured on a MH agar medium. 24 hours later, the lower concentration of the extract, where the bacteria did not thrive, was documented as the minimum lethal concentration (MBC).

2.6. Statistical analysis

A comparison of means obtained from this study was analyzed by using a two-way analysis of variance, the SPSS software version 17, and Bonferroni paired-test. A significant test level of ($P < 0.05$) was utilized to decipher the database.

3. Results

The susceptibility of standard strains to ethanol and aqueous extracts of *Rumex Alveollatus* was investigated by the disk diffusion method, the results of which are presented in Table 1. The findings of the current research reveal that the ethanolic extracts of *Rumex Alveollatus* possess an inhibitory impact on all the l researched bacteria. However, the aqueous extract did not have an antimicrobial effect, as indicated in Table 1.

Table 1 presents average diameter of the development inhibition zone of the ethanol extract of *Rumex Alveollatus* on different isolates of *Klebsiella pneumoniae*. To correlate the diameter of the development inhibition zone at concentrations of 500 to 62.5 mg/mL in *Rumex Alveollatus* ethanolic extract, a two-way analysis of variance was used. The results showed that different concentrations of ethanolic extract of *Rumex Alveollatus* had a direct effect on the lack of growth of different isolates of *Klebsiella pneumoniae* and their relationship with each other was significant (p-value=0.00). As the concentration of *Rumex Alveollatus* ethanolic extract increases, its antimicrobial activity against standard *Klebsiella pneumoniae* and its clinical isolates increases, which means that the ethanolic extract has an antibacterial effect on standard *Klebsiella pneumoniae* and its clinical isolates. No growth inhibition zone was observed at concentrations less than 62.5 mg/mL. Based on the results of the two-way analysis of variance in ethanolic extract, there is a significant difference between the diameter of the development inhibition zone at concentrations of 500 to 62.5 mg/mL and antibiotics (p=0.00). Based on the Bonferroni test, the diameter of the development inhibition zone of the ethanolic extract on the standard *Klebsiella pneumoniae* and its clinical isolates between concentrations of 50 with concentrations of 250, 125, and

62.5, concentrations of 250 with concentrations 125 and 62.5 as well as 125 with 125, were significantly different with ($p<0.05$). The three concentrations of the extract had a diameter of development inhibition zone at a concentration of 500 mg/mL relatively more than other concentrations and the diameter of a growth inhibition zone of the ethanolic extract on standard *Klebsiella pneumoniae* and clinical isolates at a concentration of 62.5 mg was the lowest among all. The minimum inhibitory concentration of ethanolic extracts of *Rumex Alveollatus* for gram-negative bacteria was 125 mg/mL and the minimum lethal concentration was 250 mg/mL.

Table 1. Mean diameter of growth inhibition zone due to antimicrobial effect of different concentrations of *Rumex Alveollatus* ethanolic extract in bacteria (in millimeters)

Concentrations (mg/mL) Sample	500	250	125	62.5	31.25
Klebsiella pneumoniae (ATCC :10031)	18.75±0.95	16.50±1.00	14.25±1.50	11.5±1.00	-
K. pneumoniae isolate 1	17.75±0.50	15.50±0.50	13.00±1.00	9.80±0.76	-
K. pneumoniae isolate 2	17.50±0.50	13.50±0.50	10.50±0.50	8.50±0.50	-
K. pneumoniae isolate 3	15.75±0.50	13.25±0.50	10.25±1.50	7.80±0.76	-
K. pneumoniae isolate 4	16.50±0.50	14.00±0.50	10.80±0.28	7.60±0.57	-
K. pneumoniae isolate 5	16.50±1.00	14.25±0.95	12.00±0.00	7.80±0.28	-

*The mean diameter of the growth inhibition zone of the antibiotic imipenem was 30 mm as a positive control

Discussion

Today, treatment with antibiotics is always a concern of the side effects of the drug. Medicinal plants with many advantages such as cheapness and availability, compatibility with nature, and better acceptance by patients, have been considered for the disease treatment, including infections. In recent years, due to the increase in microbial resistance to antibiotics and more knowledge of the side effects of synthetic chemical compounds, there is a great desire among people to use plant products with antimicrobial properties. Medicinal plants include 2,000 species of plants that are valuable resources for the preparation of healthy medicines in the world. *Rumex Alveollatus* is one of the plants that are generally when it come in traditional medicine [11,15]. The findings of the current research revealed that ethanolic extract of Rumex Alveollatus has an inhibitory effect on the development of clinical and the standard isolates of *Klebsiella pneumoniae*. The mean diameter of the development inhibition zone of *Rumex Alveollatus* ethanolic extract at concentrations of 500, 250, 125, and 62.5 on *Klebsiella pneumoniae* was 18, 16, 14, and 11 mm, respectively.

In 2017, Korkorian *et al.* examined the active ingredients and antibacterial

activity of the aqueous and ethanolic extracts of *Romex Alveollatus* on a number of gram-negative bacilli. The diameter of growth inhibition zone obtained from ethanolic leaf extract at 500 mg/mL concentration on *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella typhi* was 22.83 ± 0.76 mm. The MIC results of ethanolic extract of plant leaves and roots against *Pseudomonas aeruginosa* were 125 mg/mm. In this study, the phenolic compound of 1,2-benzene D-carboxylic acid (89.68) was the main compound isolated from *Romex alveolatus* [7]. In 2017, Pouremadi *et al.* examined the antibacterial activity of *Romex caprius* seeds on *Escherichia coli* and *Staphylococcus aureus*. In this study, ethanolic and ethanolic extracts of *Romex caprius* seeds inhibited the growth of *Staphylococcus aureus* and *Escherichia coli*. The MIC and MBC values of ethanolic and ethanolic extracts against *Escherichia coli* and *Staphylococcus aureus* were 62.5 and 125 mg/mm, respectively. In this study, 1, 2 benzene di-carboxylic acid diisoacetyl ester with 24.25% was the most isolated compound from seed [16]. In 2013, Sichani *et al.* evaluated the antibacterial activity of ethanolic and ethanolic extracts of *Rumex Alveollatus* leaves against *Pseudomonas aeruginosa*. All extracts showed a significant inhibitory effect on selected bacterial strains. There was a statistically significant relationship between antibacterial activity and concentrations of extracts. The MIC values of the extracts ranged from 31.3 mg/mL to 62.5 mg/mL [10].

In 2019, Idris *et al.* evaluated the antimicrobial activity of Rumex crispus leaf and root extracts. Acetone root extract indicates the highest antimicrobial potency (MIC less than 1.562 mg/mL) for *Klebsiella pneumoniae* [9].

In the study of Moradi *et al.* (2015), the antimicrobial effect of aqueous and ethanolic extracts of *Romex Alveollatus* on a number of prominent microorganisms was investigated by disk diffusion method. The ethanolic extract of *Romex Alveollatus* leaf at a concentration of 300 mg/mL on *Klebsiella pneumoniae* created a growth inhibition zone of 8.42 mm [17]. In Jahani study, the minimum inhibitory concentrations of *Romex Alveollatus* ethanolic extract on *Escherichia coli* (ATCC:25922) and *Pseudomonas aeruginosa* (ATCC:27853) were reported to be 50 and 25 mg/mL, respectively [18]. In Nisa study, the results of *Romanex dentatus butanol* extract indicated that at a concentration of 250 µg/mL, the growth inhibition zone for *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* was 17, 17, and 8 mm, respectively [19]. In Coruh study, the minimum inhibitory concentration of ethanolic extract of *Romex Crispus* aerial part at 10 mg/mL against Salmonella typhimurium was determined to be 62.5 mg/mL [20]. The results of the present study were in line with the studies of other researchers. Research on the extract of *Romex Alveollatus* plant has antimicrobial properties in the compounds presence such as flavonoids, glycosides, reducing sugars, tannin, anthraquinone, and alkaloids [17]. According to a study by McGigan *et al.* (2002), gram-negative bacteria are more resistant to extracts than gram-positive bacteria and may have different effects on the growth of gram-positive and gram-negative bacteria, as a result of the structural disparities between the walls of these two types of bacteria. The exterior membrane of gram-negative bacteria functions as an impediment to the entry of huge hydrophobic molecules. Likewise, the extracts had a majority of active compounds which are hydrophobic, and these elements lack the ability to infiltrate and access the active points within gram-negative bacteria, and on the other hand, they possess enzymes in the periplasmic

space that are able to remove foreign molecules. Therefore, gram-negative bacteria are usually more resistant to these compounds [21]. Low solvent toxicity, the ability to evaporate at low temperature without forming bonds with the extract's active compound or their degradation is adequate properties of the solvent in the formulation of plant extracts. Thus, hinging on the target category, which could be non-polar or polar extraction material, category of solution is likely to also vary. Most antimicrobial compounds are perfumed plants or organic compounds, primarily extracted through ethanoic and ethanolic solutions. Aqueous extracts are used in traditional medicine, but study has demonstrated that extracts formulated with organic solvents have more stability and antimicrobial effect. Because most known antimicrobial active compounds are insoluble in water, extracts from organic solvents are more potent in extracting antimicrobial compounds. Researchers have attributed the antibacterial effects of *Romex Alveollatus* to phenolic compounds. Probably for this reason, the aqueous extract does not have an antibacterial effect due to the low concentration of water-soluble phenolic compounds. This is consistent with the studies of Nisa [19] and Yildirim [22].

Conclusion

Based on the results of disk diffusion and micro-dilution investigations and comparison of these results, it can be claimed that the ethanolic extract of *Romex Alveollatus* possesses a very favorable ability to inhibit the standard growth and clinical strains of *Klebsiella pneumoniae*. Due to the significant antibacterial effects of ethanolic extracts of *Romex Alveollatus* on pathogenic bacteria, it is recommended that more extensive studies be performed *in vitro* to discern the active concentration of this extract on the desired clinical strains, bacteria, and side effects. Its precise formulation should be further evaluated and *in vivo* studies performed on laboratory mice. Then, according to the determination of the antibacterial properties of the plant extract, after confirmation of the relevant tests and issuance of a license by the Ministry of Health and Medical Education, the composition of the extract and the production of plant drops with antibacterial properties can be used instead of using chemical drugs in the treatment of some diseases.

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Conflicts of Interest

None declared.

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