# Comparison of the Antimicrobial Activity of Caucasian Wingnut Leaf Extract (*Pterocarya fraxinifolia*) and Walnut (*Juglans regia* L.) plants

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## ABSTRACT

Microbial agents are spread around us and the environment and always are dangerous for the health of animals, plants and humans. *Pterocarya fraxinifolia* L. (Juglandaceae) has biological effects and antioxidant activity. *Juglans regia* L. using natural methods associated with minimal side effects and plants with antiseptic properties or rooms is one of the natural ways. And also this plants that have biological, antimicrobial properties, and is applied in traditional medicine and due to this fact that antibacterial activities was not done on the Caucasian wingnut and walnut plants, the present study is done with the purpose of examining anti-bacterial properties. Caucasian wingnut (*Pterocarya fraxinifolia* L.) and walnut (*Juglans regia* L.) from Mazandaran province (Iran) are collected and dried. Methanol was used for extraction and it was carried out in leaf organs with percolation method. In order to do the antimicrobial tests, in wells and microdilution in tube methods, with *Klebsiella* and *Escherichia coli* species were used as the microorganisms. The growth media inoculated with the microorganisms were put for 24 hours at 37°C and the MIC and MBC values were obtained. The antimicrobial effect of the *Juglans regia* L. on *Klebsiella* was found to be better than that of the *Pterocarya fraxinifolia* L. The MIC value was 75 mg/ml and the mean diameter of the zone of inhibition was 16.33 in return 16 mm. With *E. coli* however, the opposite was found to be the case, with the *Juglans regia* L. being more potent. In this case the MIC value was found to be 75 mg/ml and the diameter of the inhibition zone was 23 in return 21.66 mm. Microdilution and well methods confirmed each other in evaluating antimicrobial activity of Caucasian wingnut and walnut plants on the *Klebsiella* and *E. coli* bacteria and first concentration (150 mg/ml), third concentration (75 mg/ml) are better between extract concentrations. Antimicrobial activity of this plant *Juglans regia* L. is better than Caucasian *Pterocarya fraxinifolia* L. Totally, significant and strong antimicrobial activity is reported from these two plants.

**Keywords**: Antimicrobial activity, Bacteria, *Pterocarya fraxinifolia*, *Juglans regia*

## INTRODUCTION

Since Human had recognized bacteria, he was always looking for effective drugs against infections caused by it and therefore achieved to effective mechanisms to eliminate antibiotics [1]. Microbial agents are spread around us and the environment and always are dangerous for the health of

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animals, plants and humans and destroy a lot of useful elements. Control of microorganisms in the living environment and in the preparation of different materials for human consumption is of great importance [2]. Gram-negative bacteria such as Vogesproskaure (positive, including *Klebsiella*) are recognized as the natural flora of the gut and mouth and can be found as Saprophyte on digestive and respiratory tract of healthy people, even babies [3]. In recent years, it has become clear that bacteria such as *Klebsiella* caused infections in many different cases and the importance of this group of organisms has been accepted as the serious infections factor in hospitalized patients [4]. *Escherichia coli* is considered as the natural inhabitants of the human and animal intestine, however it could be found in water, soil and even plants [5]. The most common bacteria, *E. coli* is one of the most important bacterial causes in the urinary tract infections and hospital opportunistic pathogens that have been isolated from human infections. Its drug resistance is of great importance especially in hospitalized patients due to this fact that beta-lactam antibiotics have become resistant. Therefore, treatment of infections caused by *E. coli* is difficult [6]. Antimicrobial resistance in *E. coli* has been reported worldwide and the rate of increasing resistance in this bacterium has created many concerns in developing and developed countries [7]. In the studies and research carried out, some herbs and medicinal plants have impact on bacteria and medicinal plants and plant extracts containing the antimicrobial effects are used [8]. Caucasian wing nut plant with the scientific name of *Pterocarya fraxinifolia* L. (Juglandaceae) is a species of tree which is thick, deciduous with fissured bark, dark gray to a height of 35 m. These species grow in the provinces of Golestan, Gilan and Mazandaran and in recent years, its small masses are found in Ilam and Lorestan provinces and also Turkey and Caucasus [9]. Despite the high prevalence, little information has been reported on this plant [10]. The reported therapeutic effect of this plant is sweat-inducing effect [11]. In a report on the screening of three medicinal herbs from Iran, methanol extract of aerial organs of *Pterocarya fraxinifolia* plant (of northern forests) in the qualitative form on the TLC plate in test of trapping free radical DPPH has showed effect + 3 from itself [12]. Walnut or *Juglans regia* L. (Juglandaceae) [13,14] leaf is used in medicine for thousands of years [15]. In the 17th century, English professionals used walnut leaf with onions and salt and honey for detoxification of snakes and scorpions and its extract was used to treat skin diseases such as tuberculosis, cervical lymph nodes, herpes, eczema and wounds [16]. In the 19th century, French were using walnut leaf in the form of syrups or injection for the treatment of cervical lymph node tuberculosis in children and also its extract was used in the form of ointment to treat wounds and sore eyes [17]. As well as, it is introduced as anti-fungal in in laboratory environment against *C. albicans* and *Trichophyton rubrum* [18]. Walnut leaf has anti-virus property and bactericide and volatile oil have fungicide activity [19]. It also has anti-cancer, blood purification and antioxidant properties [20] and different parts of walnut are used in the pharmaceutical and partly also demonstrated antimicrobial effect [21]. Many microorganisms could be destroyed or halted their growth using various chemical and physical factors. Microorganisms are often controlled creating situations that are intolerable for germs; natural products as well as herbs have fewer side effects than other chemicals products [22] and have properties and active ingredients which make them important in this respect [23]. Contact of many acids, organic alkalis, and solvents with skin lead to irritation that result in redness, swelling and burning and in advanced stages even blistering and scarring and loss of subcutaneous tissues [24]. Therefore, using natural methods associated with minimal side effects and plants with antiseptic properties or rooms is one of the natural ways. Secondary metabolites in plants are produced as stored inactive precursors in plant tissues and then released in response to environmental stresses; Starting materials in plant tissues are including glycosides, alkaloids, poly acetylene, phenol, flavonoids and flavonols which recently secondary metabolites of medicinal plants such as herbal extracts and essential oils in terms of antibacterial activity have been studied [25] and it has been specified that some derived herbal essential oils and extracts have anti-parasitic, fungal, viral as well as antibacterial properties [26]. Thus, considering the importance of the issue

and use of medicinal plants and done activities on them [27] as well as local and medical information about the numbers and appropriate forms in the medical and health sector and of course Caucasian wingnut plants which have biological properties such as pharmaceutical and antioxidant [28] and also walnut that have biological and antimicrobial properties and is applied in traditional medicine and due to the this fact that antibacterial activities was not done on the Caucasian wingnut and walnut plants and their methanol extract, the present study is done with the purpose of examining anti-microbial effect and anti-bacterial properties of methanol extracts with different concentrations in Caucasian wingnut and walnut plants and determining effective factors such as extract concentration and the initial concentration of bacteria on different species of bacteria such as *E. coli* and *Klebsiella* in wells and micro dilution in tube methods.

## MATERIALS AND METHODS

### Collection and preparation plants

Caucasian wingnut (*Pterocarya fraxinifolia* L.) and walnut (*Juglans regia* L.) from the forest of Mazandaran province (Esfivard shorab rural district, The central part of Sari, Galeh Kola Sofla, Kord Kheyl) are collected and is identified and confirmed by botanist. Gathered plants are dried in the shade and away from direct sunlight and then were split into smaller pieces [29].

### Preparation of plant extracts

Methanol was used for extraction and it was carried out in leaf organs with percolation method to have a biological effect. Dried organs were extracted by methanol solvent. Solvents were evaporated in a vacuum and were dried with the help of freeze dryer and were kept in the -80°C Deep freezer until the desired concentration is prepared [30].

### Investigating the antimicrobial effect

Condensed extracts are prepared by 10 percent solvent (DMSO - Dimethyl Sulfoxide, Merck, Germany) with 100, 150, 50, 75, 5, 37, 25 mg/ml doses for use in determination test of MIC and well diffusion. Both well and micro dilution methods were used in the pipe. Examined microorganisms including *Klebsiella* (ATCC 7881) and *E. coli* (ATCC 25922) were prepared in the lyophilized form from Microbial Collection of Tehran University. 4-5 bacteria colonies are added to the Mueller Hinton Broth Culture medium (Merck, Germany) to prepare a microbial suspension from fresh and young culture. Turbidity of prepared bacterial suspension was adjusted in accordance with the McFarland standard pipe of 0.5 (Turbidity equal to $1/5\times10^8$). Samples were diluted. In this study, the antimicrobial effect of methanol extracts were analyzed in two Agar Well Diffusion and Microdilution methods [31]. For well diffusion method, the microbial suspension of $1/5\times10^6$ *cfu/ml* on transferred Mueller Hinton agar medium was cultured and then extract concentration of 25, 37, 5, 75, 50,100,150 and 25 mg/ml were poured on the wells and DMSO of 0.5 % as the negative control and chloramphenicol antibiotics as the positive control were used to dissolve the extracts [32]. Plates were incubated at 37°C for 24 hours and after a certain period, microbial cultures were measured in millimeters in terms of form or lack of growth inhibition zone. The minimum inhibitory concentration of growth and minimum bactericidal concentration of methanol extracts with serial dilution method were determined. The last dilution where there was not any turbidity (no growth) was considered as the MIC [33]. After that all tubes with no bacterial growth were sampled and were determined through culture in minimum bactericidal MBC concentration plates. To reduce the experimental error, each of the above experiments is repeated 3

times with precise statistical methods and the average is calculated and presented. Analysis of variance and Chi-square test is used in order to evaluate significant differences in test results and significant differences between the groups are determined in the significant level of $p<0.001$ [34].

## RESULTS AND DISCUSSION

Antimicrobial property of Caucasian wingnut aerial organ and its MIC is determined in the concentration of 75 mg/ml. the MCI value in the methanol extract of white *Pterocarya fraxinifolia* L. aerial organ on the *Escherichia coli* is 75 mg/ml and MBC value is 100 mg/ml. The same is true in the case of well and the maximum diameter of the growth inhibition zone concentration is 75 with the value of 21.66±0.956 mm (Table 1).

Antimicrobial property of Caucasian wingnut aerial organ and its MIC is determined in the concentration of 100 mg/ml on the *Klebsiella*. The MCI value in the leaf methanol extract on the k is 75 mg/ml and MBC value of that concentration is more. The same is true in the case of well and the maximum diameter of the growth inhibition zone concentration is 75 with the value of 16±0.382 mm (Table 1).

Antimicrobial property of walnut aerial organ (*Juglans regia* L.) and its MIC is a better method in the concentration of 75 mg/ml. The MCI value in methanol extract of walnut leaf organ on the k is 75 mg/ml and MBC value is more. The same is true in the case of well and the maximum diameter of the growth inhibition zone is 75 with the value of 16.33±0.476 mm (Table 2).

Antimicrobial property of walnut leaf organ (*Juglans regia* L.) and its MIC is a better method in the concentration of 75 mg/ml on the *Klebsiella*. The MCI value in methanol extract of walnut aerial organ on the *Escherichia coli* is 75 mg/ml and MBC value is 100 mg/ml. The same is true in the case of well and the maximum diameter of the growth inhibition zone is 75 with the value of 23±0.956 mm (Table 2).

Table 1. The average diameter of growth inhibitory zone in standard microbial strains to the different concentrations of the Caucasian wingnut extracts (*Pterocarya fraxinifolia* L.).

| Standard microbial strain | The diameter of growth inhibitory zone in the concentration of aerial organ extracts (mg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 150 | 100 | 75 | 50 | 37.5 | 25 | P-value |
| *Escherichia coli* | 19.66± 1.73 | 19.33± 1.12 | 21.66±0.956 | 16.66± 0.654 | 13±0.456 | 12.33±0.324 | P=0.016 |
| *Klebsiella* sp. | 14.33± 0.669 | 12.33±0.574 | 16±0.382 | 11.66± 0.1895 | 9.33±0.15 | 3.66±0.150 | P=0.001 |

Table 2. The average diameter of growth inhibitory zone in standard microbial strains to the different concentrations of the walnut extracts (*Juglans regia* L.).

| Standard microbial strain | The diameter of growth inhibitory zone in the concentration of aerial organ extracts (mg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 150 | 100 | 75 | 50 | 37.5 | 25 | P-value |
| *Escherichia coli* | 20.33±1.248 | 21±1.153 | 23±0.956 | 18.33±0.654 | 16.66± 0.456 | 13±0.324 | P=0.001 |
| *Klebsiella* sp. | 15.33±0.7636 | 12.66±0.66 | 16.33± 0.476 | 11±0.2856 | 7±0.15 | 5.66±0.15 | P=0.016 |

Figure 1. A view of the effect of extracts on *E. coli*.

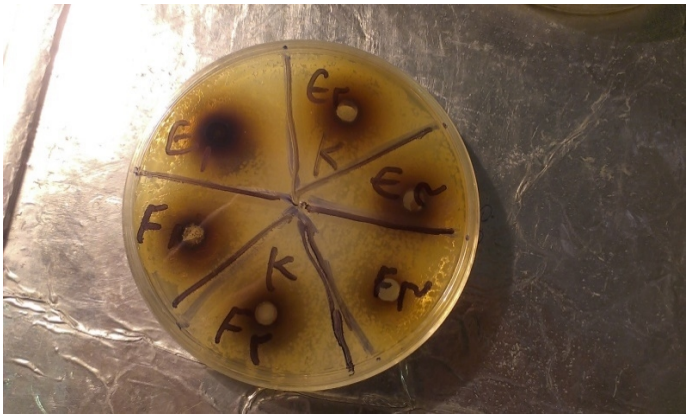
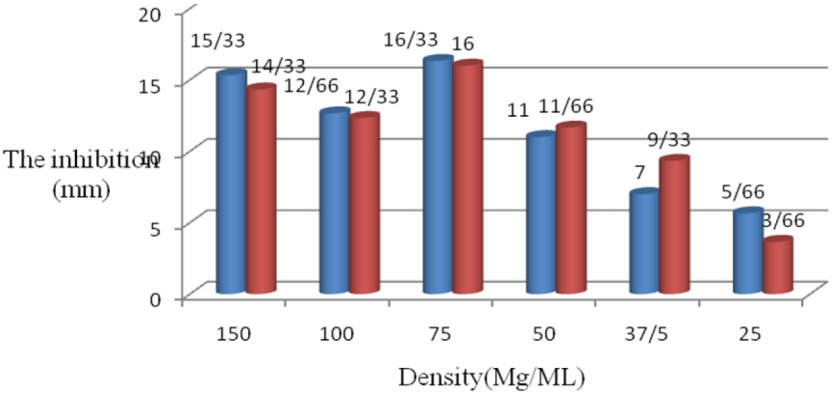
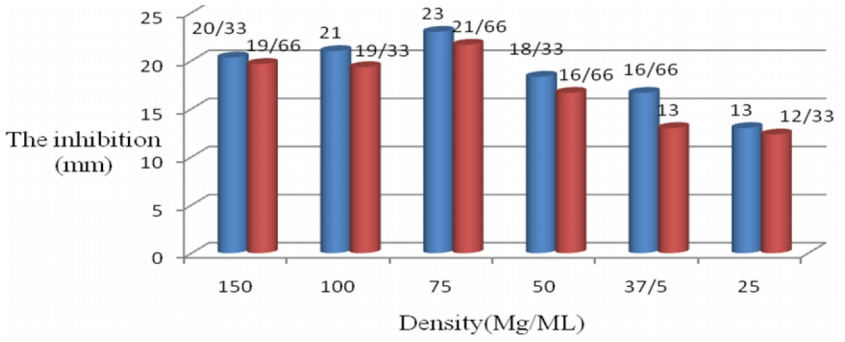


Figure 2. A view of the effect of extracts on *Klebsiella* sp.




*Juglans regia* L. , *Pterocarya fraxinifolia* L.

Figure 3. Comparing the growth inhibition zone of *Klebsiella* bacteria on leaf organ of Caucasian wingnut (*Pterocarya fraxinifolia* L.) and walnut (*Juglans regia* L.) plants.




*Juglans regia* L. , *Pterocarya fraxinifolia* L.

Figure 4. Comparing the growth inhibition zone of *E. coli* bacteria on leaf organ of Caucasian wingnut (*Pterocarya fraxinifolia* L.) and walnut (*Juglans regia* L.) plants.

Comparing and analyzing the average diameter of the growth inhibition zone in various concentrations of Caucasian wingnut plant *Pterocarya fraxinifolia* L.) and walnut (*Juglans regia* L.) on the diameter of the growth inhibition zone of *Klebsiella* bacteria (Figure 3) and *Escherichia coli* (Figure 4) showed that there is a significant difference ($p$<0.005) between the effect of different concentrations of extract in each plant on the growth inhibition zone of *Klebsiella* and *Escherichia coli* bacteria (Figure 1, Figure 2). The zone diameter of high concentration compared to low concentration is higher in ANOVA test with calculating standard deviations. The extracts were more effective on *E. coli* and its significant antimicrobial activity can be used in the case of disease and therefore, it is better to used them.

Thus, according to the desired effect of anti- bacterial *Klebsiella* and *E. coli* of Caucasian wingnut extract (*Pterocarya fraxinifolia* L.) and walnut (*Juglans regia* L.) in the present study, it can be concluded that secondary compounds in the leaves of these plants can have more effective treatment of pneumonia and urinary tract infection. The diameter of growth inhibition zone and antimicrobial effect of walnut plant (*Juglans regia* L.) compared to Caucasian wingnut extract (*Pterocarya fraxinifolia* L.) are better and more effective on the *Klebsiella* bacteria (Figure 3) and *E. coli* bacteria (Figure 4).

In a study conducted in 2014 by Jammehdar and his colleagues, the antibacterial effect of extracts of Iran native plants on the standard strain of *Pseudomonas aeruginosa* were investigated. In this study, the minimum inhibitory concentration of (MIC) in the walnut plant was 25% and in well was 17.6 mm [35]. Nabavi and colleagues in 2008 examined antioxidant activity of methanol extract of the leaf and bark of branches in Caucasian wingnut plant and concluded that in general, methanol extracts of this plant in all studied models showed different levels of antioxidant activity of their own. In particular, they showed the revival of producing Fe III to Fe II by donating electrons, chelating properties of Fe II and anti-lipid peroxidation activity [36]. Ebrahimzadeh and colleagues (2013) in their study showed that walnut had significant antihemolytic and phenol and flavonoid activity and its contents were especially quercetin. Abedigheshlaghi [37] examined the cytotoxic effect of aqueous and alcoholic extract of Caucasian wingnut plant in K562 cells. They concluded that plant was considered as a potential candidate for more studies in the treatment of CML due to the cytotoxic effects of aqueous and alcoholic extract of Caucasian wingnut plant in K562 cells [38]. Oliveira et al. (2009) examined on the antimicrobial effect of aqueous extract of walnut shell on the gram-negative, gram positive bacteria, yeast and mold. The results showed that phenolic compounds only had antimicrobial effect on gram-positive bacteria such as *Bacillus cereus*, *Staphylococcus aureus* and *Bacillus subtilis* [39]. In the present study, the antimicrobial

activity of Caucasian wingnut (*Pterocarya fraxinifolia* L.) and walnut (*Juglans regia* L.) plants on the *Klebsiella* and *E. coli* bacteria was done that the most diameter of growth inhibition zone was in walnut and on the *E. coli* bacteria with the value of 23±0.956 mm.

## CONCLUSION

Microdilution and well methods confirmed each other in evaluating antimicrobial activity of Caucasian wingnut (*Pterocarya fraxinifolia* L.) and walnut (*Juglans regia* L.) plants on the *Klebsiella* and *E. coli* bacteria and first concentration (150 mg/ml), third concentration (75 mg/ml) are better between extract concentrations. Antimicrobial activity of this plant against *E. coli* bacteria in walnut plant (*Juglans regia* L.) is better than Caucasian wingnut (*Pterocarya fraxinifolia* L.) and is the same against *Klebsiella* bacteria. Totally, significant and strong antimicrobial activity is reported from this two plants in the microdilution (tube) and well methods.

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