Available online at
http://journals.usamvcluj.ro/index.php/promediu

ProEnvironment

ProEnvironment 11 (2018) 32 - 36

Original Article

Antimicrobial Activity of Essential Oils from *Juglans regia* L. (Juglandaceae) Leaves Grown in the West Anatolian Area

OKAN Kemalcan[1], Sinem AYDIN[2], Elif APAYDIN[3], Emre SEVİNDİK[1*]

[1]*Faculty of Agriculture, Department of Agricultural Biotechnology, Adnan Menderes University, South Campus, Cakmar, Aydin, Turkey*
[2] *Giresun University, Department of Biology, Faculty of Science and Arts, Giresun, Turkey*
[3]*Giresun University, Center Research Laboratory Application and Research Center, Giresun, Turkey*

Received 13 February 2018; received and revised form 28 February; accepted 7 March
Available online 30 March

Abstract

In this study, chemical composition determination and in vitro antimicrobial effects of essential oils of *Juglans regia* plant grown under Aydın ecological conditions were evaluated by using disc diffusion methods.. Extractions were carried out with clevenger apparatus and essential oil compositions were determined by Gas Chromatography-Mass Spectrometry (GC-MS). Microorganisms used for the antimicrobial studies were *Salmonella enterica serovar typhimirium* ATCC 14028 and *Staphylococcus aureus* subsp. *aureus* ATCC 25923, *Yersinia pseudotuberculosis* ATCC 911 and *Bacillus cereus* 702 ROMA, *Enterobacter aerogenes* CCM 2531, *Bacillus subtilis* IMG 22 and *Proteus vulgaris* FMC. As a results of, essential oils obtained from the *Juglans regia* leaves plant were detected to contain 9-octadecene (17.71%), hexadecanoic acid (11.32%) and, cembrene (9.49%) at most. For the in vitro antimicrobial activity determination of essential oils, disc diffusion methods were used in our study. Furthermore, 6-17 mm zone diameters were detected in disc diffusion methods respectively. The highest resistance zone were against *P. vulgaris* and *Staphylococcus aureus* subsp. *aureus* with 17 mm diameter while the least resistance zone was detected against *Y. pseudotuberculosis*, 6 mm diameter. As a result, it is thought that the essential oil content differs in quantity and composition from the past studies, due to the different geographical and environmental effects of the plant.

Keywords: *Juglans regia, essential oil, antimicrobial, Turkey.*

1. Introduction

Medicinal plants have the capacity of inhibiting growth of widespread pathogenic bacteria due to the having essential oils they contain. The antibacterial effect of essential oils derived from medicinal plants has been verified [8, 16, 19]. Essential oils are produced by the secondary metabolism of plants.

Essential oils are natural, volatile liquid, complicated compounds which are characterized by an intense scent, seldom painted, soluble in lipid and organic solvents. They can be synthesized by all plant organs, such as stems, seeds, fruits, flowers, leaves, roots, wood or bark and twigs [1]. The Juglans (Juglandaceae) genus consists of 7 to 45 species according to a taxonomic study [28]. *Juglans regia* L. (Walnut) is the oldest culture in the world and naturally grows in almost all regions of Anatolia having suitable climatic and geographical conditions [21, 26]. Its leaves, bark and fruit are used as raw hay. Antibacterial and antifungal properties of walnut fruit extracts have been described in the literature [17, 25].

* Corresponding author.
Tel: +902567727024
Fax: +902567727233
e-mail: ph.d-emre@hotmail.com

In addition, *Juglans* leaves have been used to treat skin complaints including acne, jock itch, athlete foot, eczema, itching and varicose ulcer [4]. The aim of the present study was to determine the essential oil contents of *Juglans regia* leaves plants grown under West Anatolian ecological conditions and to investigate their antimicrobial activity on various pathogenic bacteria.

2. Material and Method

Plant Material and GC-MS Analysis

In this study, chemical composition of the essential oils *Juglans regia* (Juglandaceae) leaves were investigated. *Juglans regia* leaves of the plants were collected as study materials in June 2017 from Aydın/Koçarlı surroundings. Extractions were carried out with Clevenger apparatus and essential oil compositions were determined by Gas Chromatography-Mass Spectrometry (GC-MS). Chracterization of essential oil components was based on the library (Wiley and NIST) comparision with the mass spectra of the injected essential oil samples.

Test Bacteria

Seven bacterial strains were utilized to detect antibacterial action of the essential oils. *Salmonella enterica serovar typhimirium* ATCC 14028 and *Staphylococcus aureus* subsp. *aureus* ATCC 25923 were obtained from Giresun Province Control Labaratory, *Yersinia pseudotuberculosis ATCC 911* and *Bacillus cereus* 702 ROMA were obtained from Molecular Biology Department of Rize University. *Enterobacter aerogenes* CCM 2531, *Bacillus subtilis* IMG 22 and *Proteus vulgaris* FMC 1 were obtained from Department of Biology, Fırat University. Bacterial strains were maintained on nutrient agar at 4 °C.

Antibacterial Activity

Examination of essential oil for antibacterial efficiency was performed by the disc diffusion method. It was applied using a 24 hour culture at 37°C in 10 ml of Mueller Hinton Broth. The turbidity of bacterial suspensions were adjusted with 0.5 McFarland standard. The oils were dissolved in ethanol:hexane (1:1) by using 51% Tween 80 solution to give stock solution [15]. Dissolved extract was sterilized by using 0.45 µm pore sized filter. Sterilized nutrient agar medium was poured in petri dishes and was allowed to solidify. The bacterial suspension inoculated into Mueller Hinton agar plates. Sterile discs were put (5 mm diameter) on the agar 20 µL essential oil of *Juglans regia* and 20 µL DMSO were put on the discs, respectively. The inoculated plates were left in refrigerator for one hour then plates were incubated at 37 °C overnight [12, 22]. Diameter of inhibition zones were measured in millimeters. Inhibition zones of the essential oil were compared with standard antibiotics (tetracycline and gentamycine).

3. Results and Discussions

Essential oils, also known as volatile and ether oils, are fragrant oily liquids obtained from plant material [2]. In their compositions, there are mainly terpenoids, acids, alcohols, aldehydes, ketones, acyclic esters, lactones, and more rarely nitrogenous and sulfur compounds, coumarins and homologs of phenylpropanoids [2, 5, 7, 9, 11, 27]. In our study, as *Juglans regia* leaves essential oil composition components were given in Table 1, and gas chromatogram of the oil is shown in Fig. 1.

Table 1. Essential oil composition of *Juglans regia* leaves

RT (min)	Component	Quantity (%)
20.088	octadecanal	3.28
21.622	2-dodecene	4.84
25.185	hexadec-7-en-16-olıde	0.74
25.844	methanone	2.58
27.029	5-octadecene	6.22
27.177	oxalic acid	1.08
27.985	octadecane	0.94
28.607	tetradecanoic acid	1.22
29.659	9-octadecene	17.71
31.089	cycloheptadecanone	0.16
32.326	hexadecanoic acid	11.32
33.837	bicyclo[13.1.0]hexadecan-2-one	0.44
34.785	8-hydroxy-1-(2-hydroxyethyl)-1,2,5,5-tetramethyl-cisdecalin	2.34
35.533	octadecanoic acid	6.96
36.807	cembrene	9.49

The essential oils obtained from the *Juglans regia* leaves were detected to contain 9-octadecene (17.71%), hexadecanoic acid (11.32%) and, cembrene (9.49%) at most (Table 1). There are various research on the determination of essential oil composition obtained from walnut leaves. Bou-Abdallah et al. (2016) obtained essential oils from the walnut leaves in Mateur (north of Tunisia) through hydrodistillation method. As a result of the study, caryophyllene oxide (16.9 to 27.4%) and β-caryophyllene (4.0 to 22.5%) were detected [4].

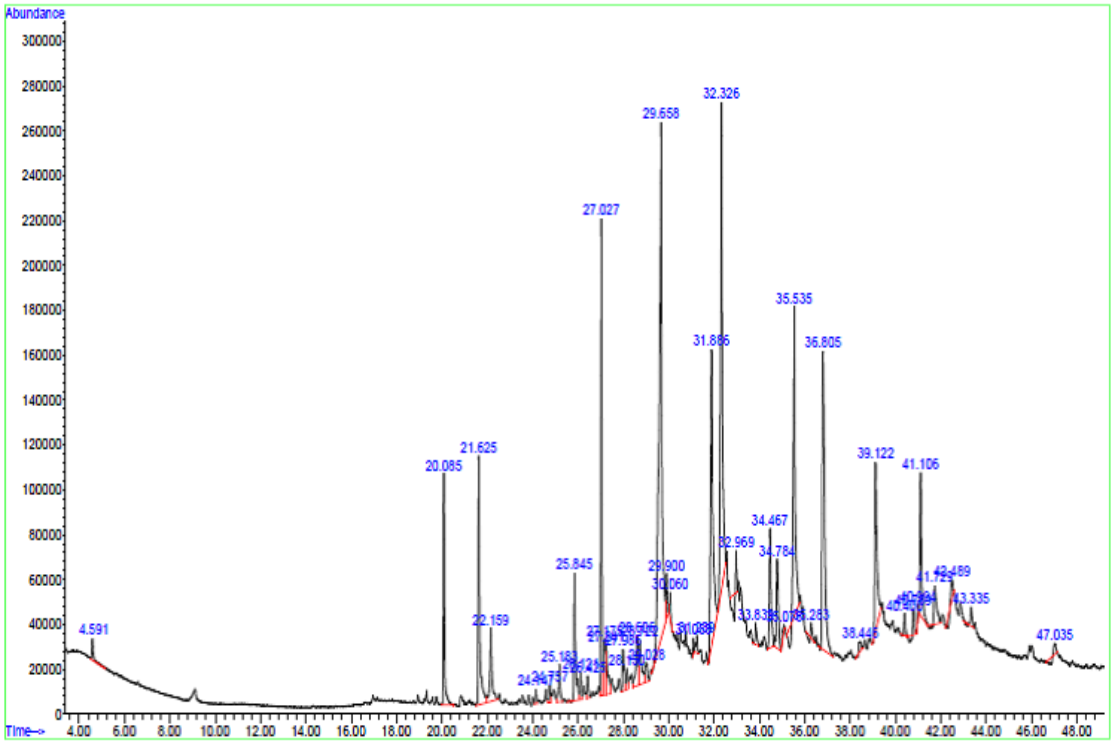

Figure 1. The gas-chromatogram of the essential oil of *Juglans regia*

Rather et al. (2012), obtained essential oils of walnut leaves from Kashmir, India through hydrodistillation method. They detected mostly α-pinene (15.1%), β-pinene (30.5%), β-caryophyllene (15.5%), germacrene D (14.4%) and limonene (3.6%) [20]. Antibiotics are the most crucial agents for inhibiting bacterial infections.

On the other hand, in recent years, these health advantages are under threat as generally utilized antibiotics have turned out less effective against certain diseases not only because of their toxic reactions, but also because of rising of drug resistant bacteria. Medicines obtained from natural sources have a key role in the protection and cure of human illnesses. The results of the antibacterial activities are demonstrated in Table 2. The results showed that *S. aureus* subsp. *aureus* and *P. vulgaris* were more sensitive when compared with the other tested bacteria species.

The growth inhibition zone measured ranged from 6–17 mm. Values <8 mm were regarded as not active against microorganisms [3].

Table 2. Inhibition zones of the essential oils of *J. regia* and antibiotics (mm)

Bacteria	Essential oil of *J. regia*	DMSO	Tetracycline	Gentamycine
S. enterica serovar typhimirium ATCC 14028 (gram -)	7	-	15	17
Y. pseudotuberculosis ATCC 911 (gram -)	6	-	-	20
E. aerogenes CCM 2531 (gram -)	13	-	11	15
B. subtilis IMG 22 (gram +)	9	-	11	16
S. aureus subsp. *aureus* ATCC 25923 (gram +)	17	-	18	19
P. vulgaris FMC 1 (gram -)	17	-	11	15
B. cereus 702 ROMA (gram +)	14	-	10	17

Gentamisin (10µg/disk); Tet: Tetrasiklin (10µg/disk)

While the highest activity was found against *P. vulgaris* (17 mm) and *S. aureus* subsp. *aureus* (17 mm), the lowest activity was observed against *Y. pseudotuberculosis* (6 mm). Among bacterial pathogens, gram positive bacterial strains were generally found to be more susceptible than gram negative bacterial strains. This is because of the fact that there are structural differences in the cell walls of gram-positive and gram-negative bacteria. DMSO (dimethyl sulphoxide) used as negative control had no activity against the bacteria. Tetracycline and gentamycine discs had higher zones than the essential

oil of *J. regia*. *J. regia* searched in this study had previously been studied for antimicrobial activities. In a study carried out by Rather et al. (2012), antibacterial activity of essential oil of the leaves of *J. regia* collected from India was screened against *Bacillus subtilis*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Shigella dyssenteriae* and *Klebsiella pneumonia*. Antimicrobial effect was found against all the tested microorganisms. In our current survey, we also found activity against *S. aureus*, *P. vulgaris*, *B. subtilis* however, essential oil of *J. regia* was inactive against *S. typhimirium* [20]. In a study conducted by Oral et al (2008) antibacterial activity of hydrosols of *J. regia* was examined against *Aeromonas hydrophila*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* [14].

Orhan et al. (2011), surveyed antimicrobial activity of the oil obtained from *J. regia* against *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Staphylococcus aureus* and *Enterococcus faecalis*. The oil inhibited all the tested bacteria. In line with this study, the essential oil which we obtained from *J. regia* inhibited to the growth of *S. aureus* [15].

Saxena et al. (2009), performed a study about the antimicrobial activity of *J. regia* oil against different test bacteria. As a result of this study, it was found that *J. regia* oil was active against *Klebsiella pneumoniae*, *Bacillus cereus*, *Staphylococcus aureus*, *Bacillus licheniformis* and *Bacillus subtilis*. Also, it was found that this oil was inactive against *Pseudomonas aeruginosa*, *Salmonella typhimirium*, *Serratia marcescens*, *Streptococcus pyogenes*, *Escherichia coli* and *Proteus hauseri* [23]. Lamichhane et al. (2016) revealed that ethanol extract of *J. regia* had an effect against *Escherichia coli* and *Staphylococcus aureus*. We also found antibacterial activity of essential oil of *J. regia* against *S. aureus* [10].

Yiğit et al. (2009) reported that water and methanol extracts of *J. regia* have antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* [29].

Qa'dan et al. (2005) screened acetone: water (7:3.5 L) extract of *J. regia*. It was found active against *Providencia stearti*, *Providencia rettgeri*, *Streptococcus* sp., *Streptococcus faeacalis* and *Staphylococcus aureus* however, it was inactive against *Proteus vulgaris*, *Escherichia coli*, *Salmonella spp*., *Pseudomonas aeruginosa* [18].

The antimicrobial feature of plant extracts may vary in accordance with the test microorganisms to be used, the extract amounts to be used against test microorganisms, plant species to be used, the plants to be collected from different localities and solvent types to be used [24].

4. Conclusions

In the present study, mostly 9-octadecene (17.71%), hexadecanoic acid (11.32%) and cembrene (9.49%) were found in the composition of essential oil obtained from *Juglans regia* leaves. It was also found that the obtained essential oil has positive antimicrobial activity results against the majority of the bacteria. As a result, these findings can be a valuable resource for further biotechnological, biological diversity, pharmaceutical and medical studies.

References

[1] Akhtar M.S., B. Degaga, T. Azam, 2014, Antimicrobial activity of essential oils extracted from medicinal plants the pathogenic Microorganisms. A review. Issues in Biological Sciences and Pharmaceutical Research, 2 (1): 001-007.

[2] Bayaz M., 2014, Esansiyel yağlar: antimikrobiyal, antioksidan ve antimutajenik aktiviteleri. Akademik Gıda, 12(3): 45-53.

[3] Bhalodia N. R., V.J. Shukla, 2011, Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: An ethnomedicinal plant. Journal of advanced pharmaceutical technology & research, 2(2): 104.

[4] Bou Abdallah, I., O. Baatour, K. Mechrgui, W. Herchi, A. Albouchi, A. Chalghoum, S. Boukhchina, 2016, Essential oil composition of walnut tree (*Juglans regia* L.) leaves from Tunisia. Journal of Essential Oil Research, 28(6): 545-550.

[5] Cowan M.M., 1999, Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12(4): 564- 582.

[6] Dobre A.A., V. Gagiu, P. Niculita, 2011, Preliminary studies on the antimicrobial activity of essential oils against Food borne bacteria and toxigenic fungi. The Annals of the University Dunarea de Jos of Galati. Fascicle VI – Food Technology, 35(2): 16-26.

[7] Dorman H.J.D., S.G. Deans, 2000, Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology, 88(2): 308-316.

[8] Encarnación M.F. R.M.N. Nolazco, R.C. López, G.R.A. Gutiérrez, S.C.G. García, C.C. Maldonado, 2016, The Antibacterial Effect of Plant-Based Essential Oils.

International Journal of Research Studies in Biosciences, 4(12): 1-6.

[9] Grassmann J., E.F. Elstner, 2003, Essential oils/properties and uses. Encyclopaedia of Food Science, Food Technology and Nutrition, (Elsevier Science Ltd.): 2177-2184.

[10] Lamichhane S., A. Hussain, M. Paudel, R.N. Jha, 2016, Phytochemical, antimicrobial and GC-MS analysis of Junglans regia Linn. Chem. Sci. Rev. Lett, 5(20): 147-156.

[11] Lawrence R., K. Lawrence, 2011, Antioxidant activity of garlic essential oil (*Allium sativum*) grown in north Indian plains. Asian Pacific Journal of Tropical Biomedicine, 1(1): 51-54.

[12] Murray P.R., E.J. Baron, M.A. Pfaller, F.C. Tenovar, R.H. Yolke, 1995, Manual of Clinical Microbiology. ASM Press, Washington D.C.

[13] Nariya P. B., N.R. Bhalodia, V.J. Shukla, R.N. Acharya, 2011, Antimicrobial and antifungal activities of *Cordia dichotoma* (Forster F.) bark extracts. Ayu, 32(4): 585.

[14] Oral N., L. Vatansever, A. Güven, M. Gülmez, 2008, Antibacterial activity of some Turkish plant hydrosols. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 14(2): 205-209.

[15] Orhan İ., B. Özçelik, B. Şener, 2011, Evaluation of antibacterial, antifungal, antiviral, and antioxidant potentials of some edible oils and their fatty acid profiles. Turkish Journal of Biology, 35: 251-258.

[16] Pattnaik S., V.R. Subramanyam, C. Kole, 1996, Antibacterial and antifungal activity of ten essential oils in vitro. Microbios, 86 (349): 237-246.

[17] Pereira J.A, I. Oliveira, A. Sousa, I.C.F.R. Ferreira, A. Bento, L. Estevinho, 2008, Bioactive properties and chemical composition of six walnut (*Juglans regia* L.) cultivars. Food Chem Toxicol, 46: 2103- 2111.

[18] Qa'dan F., A.J. Thewaini, D.A. Ali, R. Afifi, A. Elkhawad, K.Z. Matalka, 2005, The antimicrobial activities of Psidium guajava and Juglans regia leaf extracts to acne-developing organisms. The American Journal of Chinese Medicine, 33(02): 197-204.

[19] Raja S.A., M. Ashraf, A.A. Anjum, A. Javeed, T. Ijaz, A. Attiq, 2016, Antibacterial activity of essential oils extracted from medicinal plants against multi-drug resistant *Staphylococcus aureus*. The Journal of Animal&Plant Sciences, 26(2): 415-423.

[20] Rather M. A., B.A. Dar, M.Y. Dar, B.A. Wani, W.A. Shah, B.A. Bhat, M.A. Qurishi, 2012, Chemical composition, antioxidant and antibacterial activities of the leaf essential oil of *Juglans regia* L. and its constituents. Phytomedicine, 19(13): 1185-1190.

[21] Sen S.M., 1986. Walnut Growing, OMU. Press, Samsun

[22] Saric C.L.,S.I. Cabarkapa, M.B. Beljkas, C.A. Misan, B.M. Sakac, V.D. Plavsic, 2009, Antimicrobial Activity of Plant Extracts from Serbia. Food Processing Quality and Safety, 1 (2):1-5.

[23] Saxena R., D.D. Joshi, R. Singh, 2009, Chemical composition and antimicrobial activity of walnut oil. International Journal of Essential Oil Therapeutics, 3: 1-4

[24] Sesal C., G.Çobanoğlu, İ. Karaltı, B. Açıkgöz, 2016, In vitro antimicrobial potentials of four Ramalina lichen species from Turkey. Current Research in Environmental and Applied Mycology, 6(3): 202-209.

[25] Sevindik E., 2017, In silico sequence analysis of predicted beta-amylase 7-like protein in *Juglans regia* L. European Journal of Biological Research, 7(2): 148-153.

[26] Simsek M., 2016. Chemical, mineral, and fatty acid compositions of various types of walnut (*Juglans regia* L.) in Turkey. Bulgarian Chemical Communications, 48(1): 66-70.

[27] Wallace R.J., 2004. Antimicrobial properties of plant secondary metabolites. Proceeding of the Nutrition Society, 63(4): 621-629.

[28] Wani M.S., A. Hussain, S.A. Ganie, A.H. Munshi, E.P. Lal, R.C. Gupta, 2016, *Juglans regia*. A revıew. Int J Latest Res Sci Technol, 5(1): 90-97.

[29] Yiğit D., N. Yiğit, E. Aktaş, U. Özgen, 2009, Ceviz (*Junglans regia* L.) in antimikrobiyal aktivitesi. Türk Mikrobiyol Cem Dergisi, 39(1-2): 7-11.