Open Access Research Journal of
Biology and Pharmacy
Journals home page: https://oarjbp.com/
ISSN: 2782-9979 (Online)

(RESEARCH ARTICLE)

Characterization of essential oil composition of *Juglans regia* bark, comparison of secondary metabolites and biological activities of its extracts

Ayda Khadhri [1,*], Chedia Aouadhi [2], Nedaa Husein [3], Eric Masson [4] and Antonio Pizzi [5,6]

[1] *University of El-Manar II, Faculty of Sciences, Plant, Soil and Environment Interactions Laboratory, Campus Academia, 2092 Tunis, Tunisia.*
[2] *University of El-Manar II, Pasteur Institute of Tunis, Epidemiology Laboratory and Veterinary Microbiology. Bacteriology and Biotechnology Development Groups.*
[3] *Department of Allied Medical Sciences, Zarqa University College, Al-Balqa' Applied University, Jordan.*
[4] *CRITT Bois, 27 rue du Merle Blanc, BP91067, 88051 Epinal Cedex 9, France.*
[5] *LERMAB, University of Lorraine, Epinal, France.*
[6] *Department. of Physics, King Abdulaziz University, Jeddah, Saudi Arabia.*

Open Access Research Journal of Biology and Pharmacy, 2022, 04(01), 060–068

Publication history: Received on 20 January 2022; revised on 12 February 2022; accepted on 14 February 2022

Article DOI: https://doi.org/10.53022/oarjbp.2022.4.1.0025

Abstract

Aromatic and medicinal plants form a reservoir of active molecules; among them, *Juglans regia* is a plant of the *Juglandaceae* family, and it is used thanks to its medicinal properties. The present study was conducted to investigate the secondary metabolite content and biological activities of the infusion, methanolic extracts, and essential oil of the bark essential oil (EO) of *Juglans regia* harvested from North Tunisia, as well as its chemical composition.

Essential oil, infusion, and methanolic extracts were prepared. Identification of essential oil composition was determined using GC-MS. Phenolic content, antioxidant, and antimicrobial activities were evaluated with the colorimetric method.

The obtained data indicate that *J. regia* is very abundant in bioactive compounds. The infusion extract of *J. regia* bark has been found to contain elevated amounts of phenolic compounds. The infusion extract showed stronger antioxidants and radical scavenging action than methanol. The studied methanolic extract displayed antimicrobial activity against all ten tested microbial strains but with variable degrees according to the microbial strain. The infusion extract, instead, was inactive. The use of gas chromatography coupled with mass spectrometry (GC-MS) revealed the existence of 34 components in the bark (EO). The main compounds were: carvacrol (13.82%), isoeugenol (13.71%), *β*-caryophyllene (7.81%), 1,8-cineole (8.60%), and bornyl acetate (7.13%).

Our results suggest that *Juglans regia* is a natural source of potent antioxidants and antimicrobials and may be useful as preventive agents in some diseases.

Keywords: Antioxidant; Antimicrobial; Bark; Essential oil; *Juglans regia*; Polyphenols

1. Introduction

In the order of Fagales, the *Juglandaceae* are classified as the family of walnuts. Different walnut family members have large aromatic leaves with a distinct fruiting structure [1]. While mainly valued for its nutritive value as a main world nut crop, the leaves of the trees are reported to exhibit medicinal properties. The antioxidant activity of walnut liqueur,

* Corresponding author: Ayda Khadhri
University of El-Manar II, Faculty of Sciences, Plant, Soil and Environment Interactions Laboratory, Campus Academia, 2092 Tunis, Tunisia.

derived from green walnuts, is also associated with its polyphenolic composition [2]. Regarding its therapeutic benefits, *Juglan* leaves were used to treat skin complaints including acne, jock itch, athlete's foot, and eczema [3-5]

The antimicrobial and antioxidant activities of this *Juglandaceae* have also been described by Miraliakbari and Shahidi [6]. The safety advantages of walnuts are generally due to their chemical nature.

Walnut barks have been used in folk medicine for the treatment of skin inflammation, hyperhidrosis, and ulcers [7-8]. These actions are due to the astringent influence of tannin, which makes up to 10% of walnut leaves. The leaf essential oil contains monoterpenes and sesquiterpenes. As for monoterpenes, they are represented by (E)-β-ocimene (12%), β-pinene (11%), limonene (10%), with traces of sabinene, α-pinene, myrcene, and linalool, whereas sesquiterpenes by caryophyllene (15%), germacrene D (13%), with minor amounts of *(E)*-β-farnesene, and α-farnesene [9]. Nahrstedt et al*.* [10] identified 26 terpenoids from steam-distilled leaves of European walnut. The substances identified were: 21 monoterpenes, two sesquiterpenes, one diterpene, two compounds of terpenoid origin, and eugenol. The steam-distilled fraction is composed of sesquiterpenes and substances derived from esters of fatty acids, with the main substance being β-eudesmol.

Liu et al. [11] analyzed an essential oil extracted from *J. regia* leaves by hydrodistillation. Using a gas chromatography-mass spectrometry (GC-MS) method, they identified 20 components: terpenoids (84.89%), aromatic compounds (3.9%), and esters (1.34%).

Reports on the study of *Juglans* showed the presence of terpenoids [12], diarylheptanoids [13], naphthaquinones [14-15], tetralones [15], flavonoids [16], and lignans [17], which may be a suitable chemical composition for various therapeutic effects.

Lin et al. [18] were isolated from the fresh pericarps of *J. hopeiensis,* two new triterpenoids, jughopanes A and jughopanes B.

One new diarylheptanoid (3S)-3′, 4″-epoxy-1-(4′-hydroxyl phenyl)- 7-(3″-hydroxyl phenyl) heptane-3-hydroxy, together with eleven known ones, was isolated from the fresh pericarps of *J. sigillata* [19].

A new 1,4-napthoquinone derivative, namely, (S)-(-)-3-(8-hydroxy-1,4- dioxo-1,4-dihydro-naphthalen-2-yl)-3-(4-hydroxy-3-methoxyphenyl)- propionic acid methyl ester, was isolated from the roots of *J. mandshurica* Maxim [20]. From the immature exocarps of *J. mandshurica*, three new juglones were isolated among the five known tetralone derivatives, which are the key constituents responsible for the antimicrobial and cytotoxic effects [21]. One new a-ditetralonyl glucoside was isolated from the green walnut husk of *J. mandshurica*, together with twelve recognized compounds [22].

In this work, the phenolic content of infusions and methanolic extracts obtained from the barks of *J. regia* originated from North Tunisia were assessed. The antioxidant and antimicrobial activities of such extracts were evaluated. Furthermore, the chemical composition of the essential oil of *J. regia* bark was determined. The objective of this study was to discover new potential sources for the food industry of bioactive compounds and antimicrobial agents.

2. Material and methods

2.1. Plant Material

Juglans regia barks were collected (200 g) during summer, July 2013, in Jendouba (36°50'72.26"N, 08'77'56.56"E), Northern Tunisia, and identified according to the ''Flore de la Tunisie'' [23]. . The barks were dried at room temperature. Voucher specimens (JUG-J.reg/01) were preserved in our laboratory for further reference.

2.2. Preparation of Plant Extracts

Infusion extract was prepared as follows: in 100 mL of boiling water, 10g of bark was added. In a closed glass or porcelain jar, the mixture is left to rest for fifteen minutes and shaken periodically. Then, the solvent is drained through the filter paper, then freeze-dried, and lyophilized.

Methanolic extract was prepared by combining five grams of *J. regia* bark with 50 ml of methanol (80%) (3 times). After mixing for 30 min, the extract was left to rest for 24 hours at 4°C in darkness and then filtered through ashless filter

paper (Whatman No. 4). The extracts obtained were concentrated under a vacuum at 40° C and the residues were stored at 4 °C for assays.

The EO of *J. regia* barks was obtained by hydrodistillation using a Clevenger-type apparatus for 3 h. 100g of dried bark was mixed with 600 mL of distilled water. The EO yield obtained from *J. regia* bark was 0.01% (v/w). EO was collected directly from the distillate and stored in dark vials at 4 °C.

2.3. Identification of Essential Oil

The EO was analyzed by gas chromatography coupled with mass spectrometry (GC-MS) using an HP 5975C mass spectrometer (Agilent Technologies) with electron impact ionization (70 eV) [24]. The identification of oil components was assigned by comparison of their retention indices relative to (C_8–C_{22}) *n*-alkanes with those of the literature or with those of authentic compounds available in the authors' laboratory [25]. The determination of the percentage composition was based on peak area normalization without using correction factors.

2.4. Determination of Phenolic Compound Amounts

Total phenolics were assayed using the Folin-Ciocalteu reagent and total flavonoids were determined by a colorimetric assay according to Dewanto et al. [26]. The analysis of condensed tannins was carried out according to the method previously described by Sun et al. [27].

2.5. Antioxidant Activity

Three classic tests were used in this work to evaluate the antioxidant activity of different extracts: a test of radical scavenging 2,2 diphenyl-1-picrylhydrazyl (DPPH), the reducing power of iron, and iron-chelating power. The electron donation ability of the obtained extracts was measured by bleaching the purple-colored solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hatano et al. [28]. The reducing power and the ferrous ion-chelating ability were determined according to the methods of Oyaizu [29] and Decker and Welch [30], respectively.

2.6. Antimicrobial Activity

Tunisian infusion and methanolic extracts of *J. regia* were screened for their antibacterial and antifungal activities against 10 microbial strains: *Bacillus cereus* ATCC 1247, *Escherichia coli* ATCC 8739, *Listeria monocytogenes* ATCC 7644, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213, *Salmonella typhimurium* NCTC 6017, *Aeromonas hydrophila* EI, *Aspergillus niger* and *Aspergillus flavus* and *Candida albicans* ATCC 2091. Bacterial strains were cultured on trypto-caseine soja agar (TSA) and incubated at 37°C for 24h. Fungal species were cultured on a potato dextrose agar (PDA) plate at 28°C for 72h. *Candida albicans* was grown on a sabouraud dextrose agar (SDA) plate at 30°C for 48 h.

The antibacterial and antifungal activities of the studied extracts were first evaluated by the disc diffusion method [31]. Thus, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using a broth dilution method as described by Aouadhi et al. [31].

2.7. Statistical Analysis

Data were analyzed using the SAS v. 9.1.3 program (SAS, 1990). Analysis of variance (ANOVA) and Duncan's multiple range approach was used to compare any significant differences between solvents and samples. Values have been reported as means ± standard deviation. Differences were considered significant at $P < 0.05$. All analyses were carried out in triplicate and the values were the average of three replicates.

3. Results and discussion

3.1. Chemical Composition and Yield of the Essential Oil

The EO yield extracted from *J. regia* bark based on dry weight was 0.01% (v/w). The chemical constituents of bark essential oil extracted from *J. regia* were investigated by GC/MS (Table 1). The components were identified in relation to (C_8-C_{22}) *n*-alkanes by their percentage and their retention indices (RI). In the order of their elution on the HP-5 column, they are listed in Table 1.

Table 1 Chemical composition of essential oil from *J. regia*

Retention Time (i)	Retention indices (ii)	RI (iii)	Compounds	Percentage (%)
11.89	933	939	α-pinene	2±0.01
12.34	950	954	camphene	2.08±0.02
13.08	978	979	β-pinene	1.09±0.01
14.28	1027	1026	β-cymene	1.44±0.02
14.46	1035	1031	1,8-cineole	8.61±0.04
15.05	1056	1033	4-carene	0.11±0.00
16.02	1102	1099	β-linalool	2.29±0.02
16.45	1123	1114	β- thujone	0.18±0.00
16.96	1148	1139	L-pinocarveol	0.69±0.01
17.10	1154	1146	(-) camphor	2.92±0.02
17.45	1171	1153	menthone	0.65±0.00
17.63	1180	1169	borneol	1.60±0.01
17.77	1187	1177	4-terpineol	1.87±0.01
17.92	1194	1180	p-cymen-8-ol	0.46±0.00
18.08	1202	1189	α-terpineol	2.09±0.01
18.36	1216	1205	verbenone	0.33±0.00
18.64	1231	1226	β-citronellol	5.35±0.03
19.10	1254	1253	trans-geraniol	1.95±0.01
19.77	1289	1289	bornyl acetate	7.13±0.03
19.90	1296	1291	p-cymen-2-ol	1.31±0.02
20.09	1306	1299	carvacrol	13.83±0.05
21.06	1360	1369	isoeugenol	13.71±0.07
22.26	1429	1409	β-caryophyllene	7.82±0.01
22.57	1447	1443	farnesol	1.84±0.02
22.87	1465	1444	ocimene	0.58±0.01
22.94	1469	1460	alloaromadendrene	0.53±0.01
23.43	1498	1506	α-farnesene	0.53±0.01
23.84	1524	1480	δ-muurolene	1.67±0.01
24.89	1590	1578	(-) spathulenol	2.71±0.02
24.99	1596	1583	caryophyllene oxide	2.26±0.01
25.61	1637	1608	β-eudesmene	0.97±0.00
25.72	1644	1624	δ-eudesmol	1.61±0.01
25.85	1653	1654	δ-cadinol	1.28±0.01
26.11	1670	1660	β-selinenol	6.19±0.02
27.26	1748	1792	patchulane	0.29±0.00

(i) : Compounds are listed in order of their elution from an HP-5MS column; (ii): Retention index determined; (iii): Retention index reported from literature (Adams, 2001); ±: Standard deviation, results are the means standard deviations of three independent experiments.; Monoterpenes Hydrocarbons (7%); Oxygenated monoterpenes (64.98%); Sesquiterpenes hydrocarbons (11.71%); Oxygenated sesquiterpenes (16.87%).

A total of 35 compounds were identified in this oil, representing 100% of the total essential oil. Their major constituents were carvacrol (13.83%); isoeugenol (13.71%); 1,8-cineole (8.61%); β-caryophyllene (7.82%) and bornyl acetate (7.13%). The chemical classes of J. regia bark EO components were recorded in Table 2. The chemical ingredients were separated into four classes, which are hydrocarbon monoterpenes, oxygenated monoterpenes, hydrocarbon sesquiterpene, and oxygenated sesquiterpene. The main chemical constituents appeared to be oxygenated monoterpenes (64.98%).

Our findings differ from those reported by Noumi et al. [32]. These authors observed that the major constituents of *J. regia* oil were mostly composed of the aromatics eugenol (27.5%) and methyl salicylate (16.2%) as well as the sesquiterpenes, germacrene D (21.4%), *(E)*-b-farnesene (8.2%), and *(E, E)*-α-farnesene (2.6%). Smaller quantities of geranyl acetate (1.6%), linalool (1.5%), myrtenal (1.0%), and α-terpineol (1.0%) were also identified. The chemical composition of the walnut depends on the organ, location, and environmental growing conditions (climatical, seasonal, geographical), genetic differences, and the provenance of the plant, as well as distillation and storage conditions [33].

Several isolated phenolic compounds from *J. regia* such as pyrogallol, p-hydroxybenzoic acid, vanillic acid, ethyl gallate, protocatechuic acid, gallic acid, 3,4,8,9,10-pentahydroxydibenzo pyran-6-one, tannins, glansrins, adenosine, adenine, could provide a chemical source for some of the health benefits claimed for *J. regia* in diet and folk medicine [34].

3.2. Phenolic Contents

Total polyphenols, flavonoids and condensed tannin contents of infusion and methanolic extracts of *J. regia* bark were determined.

Table 2 Contents of polyphenols, flavonoids, and tannins in infusion and methanolic extracts of J. regia barks

	Total phenolic content [mg GAE/g DW]	**Flavonoid content [mg CE/g DW]**	**Condensed tannin content [mg CE/g DW]**
Methanolic extract	18.9±0.02	0.32±0.01	1.2±0.01
Infusion extract	28.8±0.06	0.44±0.03	3.6±0.02

GAE, gallic acid equivalents; DW, dry weight of extract; CE, (+)-catechin equivalent. Results are the means standard deviations of three independent experiments.

As shown in Table 2, a significant variation in the phenolic levels of the extracts was observed. The infusion extract contained a high proportion of polyphenols (28.8 mg GAE/g DW) followed by a low flavonoids fraction (0.44 mg CE/g DW) and condensed tannin (3.6 mg CE/g DW). Moreover, the total phenolic content was significantly higher in the infusion extract compared to the methanolic ones (18.9 mg GAE/g DW). Based on these results, it can be stated that the increase in the polarity of the extraction solvent, passing from methanol to water, enhanced the polyphenol content of the infusion.

Comparing our results with those obtained by Moghaddam et al. [35], it can be concluded that Tunisian *J. regia* (bark) contained a lower proportion of phenols. This difference could be attributed to genetic and geographical causes.

3.3. Antioxidant Activity

Results are shown in Table 3 summarize the variation of the concentration corresponding to 50% inhibition (IC_{50}) of the methanolic and infusion extracts of *J. regia* bark. Based on the obtained data, it can be concluded that the antiradical activity depends on the nature of the extract. The best activity was more marked for the infusion extract (IC_{50} 1.27 mg/mL).

Table 3 Antioxidant activity, Iron chelating power and Iron reducing power of the methanolic and infusion extracts of *J. regia*

Antioxidant capacity IC_{50} (mg/mL)			
	DPPH	Reducing power	Iron chelating
Methanolic extract	3.4± 0.04	0.23 ±0.02	0.41±0.01
Infusion extract	1.27±0.03	0.018±0.00	0.027±0.00

Results are the means standard deviations of three independent experiments.

The effective concentration (EC_{50}) was used to estimate the iron-reducing power. The EC_{50} values are obtained from the linear regression line between the concentration of the extract and the corresponding optical density. The low reducing power of the extract corresponds to a high EC_{50} value. Thus, from Table 3, it can be concluded that the bark infusion has the highest reducing power (0.018 mg/mL).

To assess the ability of the plant extracts to chelate metal ions, iron-chelating power was used. Table 3 illustrates the capacity of methanolic and infusion extracts of *J. regia* to chelate iron. The infusion extract had the lowest IC_{50} (0.027 mg/mL) and so the highest chelating power. All extracts showed strong antioxidant activity [36]. The results obtained showed that the infusion extract exhibited the highest antioxidant capacity compared to methanolic ones. This strong antioxidant activity of walnut bark might be attributed to the high amount of total phenols (18.9 – 28.8 mg GAE/g DW) (Table 3). The Tunisian walnut bark extracts exhibited higher antioxidant capacity compared to Iranian walnuts [35].

3.4. Antimicrobial Activity

The *in vitro* antimicrobial activities of *J. regia* extracts against Gram-positive and Gram-negative bacteria, two fungi, and yeast were evaluated. The diameter of inhibition zones, MIC, and MBC values were considered for assessing the potency of the tested extracts (Table 4). Infusion extract was indeed inactive against the tested microbial strains while the methanolic extract had the potential for antibacterial and antifungal activities against all the evaluated microorganisms. *A. hydrophila* and *P. aeruginosa* showed susceptibility with an inhibition zone of 15 mm. The intermediate effect was observed against *Bacillus cereus*, *E. coli*, and *S. typhimurium* with an inhibition zone diameter of 13 mm. The inhibition zone diameters for *L. monocytogenes* and *S. aureus* were less than 13 mm but still noteworthy. Furthermore, the inhibition zone of *A. flavus* 12 mm was superior to 11 mm of amphotericin B.

Table 4 Antimicrobial activity of methanolic extract of *J. regia*

	Inhibition zone diameters (mm)		**MIC**	**MBC**
	Extract	**Antibiotics**	**(mg/mL)**	**(mg/mL)**
Gram negative bacteria				
Escherichia coli ATCC8739	13±0.5	24[1]	0.25	0.5
Salmonella typhimurium NCTC6017	13±1	23[1]	0.25	0.5
Aeromonas hydrophila EI	15±0.7	23[1]	0.25	0.5
Pseudomonas aeruginosa ATCC27853	15±0.8	21[1]	0.25	0.5
Gram positive bacteria				
Staphylococcus aureus ATCC29213	12±0.9	20[1]	0.125	0.25
Listeria monocytogenes ATCC7644	11±0.5	18[1]	0.25	0.5
Bacillus cereus ATCC1247	13±0.5	21[1]	0.25	0.5
Fungus				
Aspergillus flavus	12±1.1	11[2]	0.25	0.5
Aspergillus niger	13±0.9	12[2]	0.25	0.5
Yeast				
Candida albicans	13±0.5	17[2]	0.25	0.5

[1]Gentamicin, [2]Amphotericin, results are the means of standard deviations of three independent experiments, ±: standard deviation

In addition, the MIC and MBC values for different bacteria strains ranged from 0.125 to 0.25 mg/mL and 0.25 to 0.50 mg/mL, respectively.

Our results corroborate previous reports about the antibacterial and antifungal potentials of *J. regia* extract. Hot and cold solvents and aqueous extracts of leaves, bark, fruits, and green husks of *J. regia* from different countries have revealed broad-spectrum antibacterial activity against *Bacillus cereus, Escherichia coli, Klebsiella pneumoniae,*

Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, and *Proteus* sp. using both agar streak and disc diffusion methods [37, 38].

Aqueous and solvent extracts of *J. regia* fruits, leaves, and bark displayed antifungal activity against a wide range of fungi using the disc diffusion method, agar dilution method, agar streak dilution and the Raddish method. Pereira et al. [39] reported that all walnut varieties exhibited antifungal activity against *Candida albicans* and *Cryptococcus neoformans* when soxhleted with light petroleum ether.

4. Conclusion

The presence of biologically active molecules as major components in the bark of *J. regia* oil makes it of great interest for medical purposes. Moreover, the finding of this work confirms the view that some aromatic and medicinal plants are promising sources of potent antioxidants and may be useful as preventive agents in some diseases.

Compliance with ethical standards

Acknowledgments

The authors acknowledge the funding supported by Campus France as part of the Scientific Stay at High Level received by A.K and supported by CRITTBois and LERMAB of the University of Lorraine, Epinal, France.

Disclosure of conflict of interest

The authors confirm that this article content has no conflict of interest.

Statement of ethical approval

No Animals/humans were used for the studies that are the base of this research.

References

[1] Elias TS. The genera of Juglandaceae in the southeastern United States. J Arnold Arbor. 1972; 53: 26-51.

[2] Alamprese C, Pompei C, Scaramuzzi F. Characterization and antioxidant activity of nocinoliquer. Food Chem. 2005; 90(4): 495-502.

[3] Felter HW, Lloyd JU. King's American Dispensatory. 18th ed., 3rd rev., Portland, OR. 1983.

[4] Bown D. Encyclopedia of Herbs and Their Uses.145. DK Publishing, Inc., New York, NY. 1995; 198.

[5] Qa'dan F, Thewaini AJ, Ali DA, Afifi R, Elkhawad A, Matalka K. The antimicrobial activities of Psidium guajava and Juglans regia leaf extracts to acne-developing organisms. The A. J. of Chinese Med. 2005; 33: 197-204.

[6] Miraliakbari H, Shahidi F. Oxidative stability of tree nut oils. J. Agric. Food. Chem. 2008; 56: 4751-4759.

[7] Bruneton J. Pharmacognosie, phytochimie, plantes medicinales. Paris: Tec & Doc. 1999.

[8] Proenca da Cunha A, Silva AO, Roque OR. Plantas e produtos vegetaisem fitoterapia. Lisboa: Fundaca˜o Calouste Gulbenkian. 2003.

[9] Buttery Ron G, Flath Robert A, Mon Thomas R, Ling Louisa C. Identification of germacrene D in walnut and fig leaf volatiles. J. Agric. Food Chem. 1986; 34(5): 820-822.

[10] Nahrstedt A, Vetter U, Hammerschmidt FJ. Composition of the steam distillation product from the leaves of Juglans regia. Planta Medica. 1981; 42: 313-332.

[11] Liu L, Li W, Koike K, Zhang S, Nikaido T. New alpha tetra lonyl glucosides from the fruit of Juglans mandshurica. Chem. Pharm. Bull. Tokyo. 2004; 52: 566-569.

[12] Hou SB, Tan TX, Du WX, Chen G. Chemical constituents from the bark of Juglans mandshurica Maxim. and their phenol oxidase inhibitory effects. Arch Phytopathol Plant Protect. 2017; 50(9-10): 1-10.

[13] Jin M, Diao S, Zhang C, Cao S, Sun J, Li R, Jiang Z, Zheng M, Son JK, Li G. Two new diarylheptanoids isolated from the roots of Juglans mandshurica. Nat Prod Res. 2015; 29(19): 18-39.

[14] Yu HY, Li X, Meng FY, Pi HF, Zhang P, Ruan HL. Naphthoquinones from the root barks of Juglans cathayensis Dode. J Asian Nat Prod Res. 2011; 13(7): 581-587.

[15] Zhou Y, Yang B, Jiang Y, Liu Z, Liu Y, Wang X, Kuang H. Studies on cytotoxic activity against HepG-2 cells of naphthoquinones from green walnut husks of Juglans mandshurica Maxim. Molecules. 2015; 20(9): 15572-15588.

[16] Si CL, Zhang Y, Zhu ZY, Liu SC. Chemical constituents with antioxidant activity from the pericarps of Juglans sigillata. Chem Nat Compd. 2011; 47(3): 442-445.

[17] Park S, Kim N, Yoo G, Kim SN, Kwon HJ, Jung K, Oh DC, Lee YH, Kim SH. Phenolics and neolignans isolated from the fruits of Juglans mandshurica Maxim. and their effects on lipolysis in adipocytes. Phytochemistry. 2017; 137: 87-93.

[18] Lin Y, Peng X, Chen J, Zhou J, Ruan H. Triterpenoids from the fresh pericarps of Juglans hopeiensis. Nat Prod Res. 2021; 35(2): 228-235.

[19] Liang J, Peng X, Zhou J, Zhou M, Ruan H. Diarylheptanoids from the fresh pericarps of Juglans sigillata. Produits naturels. 2018; 2(20): 2457-2463.

[20] Jiang Z, Diao S, Li R, Zhou W, Sun J, Zhou Y, Jin Y, Jin M, Li G. One new 1,4-napthoquinone derivative from the roots of Juglans mandshurica. Nat Prod Res. 2018; 32(9): 1017-1021.

[21] Yang Q, Yao QS, Kuang Y, Zhang YZ, Feng LL, Zhang L, Guo L, Xie ZP, Zhang SM. Antimicrobial and cytotoxic juglones from the immature exocarps of Juglans mandshurica. Nat Prod Res. 2018; 33(22): 3203-3209.

[22] Wang AD, Xie XY, Zeng WM, Liu JY, Xu YN. New α-ditetralonyl glucoside from the green walnut husk of Juglans mandshurica. Nat Prod Res. 2020; 34(21): 3066-3072.

[23] Pottier-Alapetite G. Flore de la Tunisie. Angiospermes - Dicotylédones. Vol. I: Apétales Dialypétales. Ministère de l'Enseignement Supérieur et de la Recherche Scientifique et le Ministère de l'Agriculture. Tunis, Tunisie. 1979.

[24] Bannour M, Aouadhi C, Khalfaoui H, Aschi-Smiti S, Khadhri A. Barks essential oil, secondary metabolites and biological activities of four organs of Tunisian Calligonum azel Maire. Chem. Biodiversity. 2016; 13: 1527-1536.

[25] Adams RP. Identification of essential oils components by Gas Chromatography/quadrupole Mass Spectroscopy. Carol Stream, IL, USA: Allured Publishing Corporation. 2001.

[26] Dewanto V, Wu X, Adom KK, Liu RH. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J Agric Food Chem. 2002; 50(10): 3010-3014.

[27] Sun B, Ricardo-da-Silva JM, Spranger I. Critical factors of vanillin assay for catechins and proanthocyanidins. J. Agric. Food Chem. 1998; 46: 4267-4274.

[28] Hatano T, Kagawa H, Yasuhara T, Okuda T. Two new flavonoids and other constituents in licorice root their relative astringency and radical scavenging effect. Chem Pharm. Bulletin. 1988; 36(6): 2090-2097.

[29] Oyaizu M. Studies on products of the browning reaction prepared from glucose amine. Japanese. J Nutr Diet. 1986; 44(6): 307-315.

[30] Decker EA, Welch B. Role of ferritin as lipid oxidation catalyst in muscle. Food J Agric Food Chem. 1990; 38(3): 674-677.

[31] Aouadhi C, Ghazghazi H, Hasnaoui B, Maaroufi A. Secondary metabolite, antioxidant and antibacterial activities of Teucrium polium L. methanolic extract. Int J Agric Plant Pro. 2013; 4(8): 1790-1797.

[32] Noumi E, Snoussi M, Trabelsi N, Hajlaoui H, Ksouri R, Valentin E, Bakhrouf A. Antibacterial, anticandidal and antioxidant activities of Salvadora persica and Juglans regia L. extracts. J. Med. Plants Res. 2011; 5: 4138-4146.

[33] Perry NB, Anderson RE, Brennan NJ, Douglas MH, Heaney AJ, McGimpsey JA, Smallfield BM. Essential Oils from Dalmation Sage (Salvia officinalis L.): variations among individuals, plant parts, seasons and sites. J. Agr. Food Chem. 1999; 47(5): 2048-2054.

[34] Zhang J, Jun-xi L, Fei Z, Duo-long D. Chemical constituents in green walnut husks of Juglans regia. Chin. Tradit. Herbal Drugs. 2009a; 06.

[35] Moghaddam PZ, Mohammadi A, Feyzi P, Alesheikh P. *In vitro* antioxidant and antibacterial activity of various extracts from exocarps and endocarps of walnut. Pak. J. Pharm. Sci. 2017; 30(5): 1725-1731.

[36] Qamar W, Sultana S. Polyphenols from Juglans regia L. (Walnut) kernel modulate cigarette smoke extract induced acute inflammation, oxidative stress and lung injury in Wistar rats. Hum. Exp. Toxicol. 2011; 30: 499-506.

[37] Citoglu GS, Altanlar N. Antimicrobial activity of some plants used in folk medicine. J. Fac. Pharm. Ankara. 2003; 32: 159-163.

[38] Poyrazolu EC, Biyik H. Antimicrobial activity of the ethanol extracts of some plants natural growing in Aydin, Turkey. Afr. J. Microbiol. Res. 2010; 4: 2318-2323.

[39] Pereira JA, Oliveira I, Sousa A, Ferreira IC, Bento A, Estevinho L. Bioactive properties and chemical composition of six walnut (Juglans regia L.) cultivars. Food Chem. Toxicol. 2008; 46(6): 2103-2111.