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Volatile fraction composition and biological activities of the leaves, bark and fruits of Caucasian wingnut from Iran

M. Akhbari[a]*, S. Tavakoli[a] and M.R. Delnavazi[b]

[a]*Essential Oil Research Institute, University of Kashan, Kashan, Iran;* [b]*Faculty of Pharmacy, Department of Pharmacognosy, Tehran University of Medical Sciences, Tehran, Iran*

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As a result of GC/MS and GC/FID analysis of the volatile fractions obtained from the leaves, bark, and fruits of Caucasian wingnut (*Pterocarya fraxinifolia* L.), 22, 31, and 33 compounds were identified in which aromadendrene (26.3%), biotol (37.8%), and caryophyllene (15.2%) were the main constituents of these oils, respectively. Antioxidant activities were evaluated using DPPH and β-carotene/linoleic acid assays and total phenolis contents of extracts were measured using Folin–Ciocalteu method. Among the extracts, bark extract had highest amount of total phenolic content ($\sim$179.5 μg mg^{-1}) and highest free radical scavenging activity (IC_{50}=$\sim$17.9 μg mL^{-1}) in DPPH assay and leaves extract showed highest antioxidant capacity (84.1% inhibition) in β-carotene/linoleic acid test. All the three extracts showed moderate antibacterial activities against *S. dysenteriae* and all volatile fractions showed potent antimicrobial activities against *S. epidermidis* in both disc diffusion and micro-well dilution tests. The examined samples had low cytotoxic effects based on brine-shrimp lethality assay.

Keywords: Caucasian wingnut; *Pterocarya fraxinifolia* L.; volatile fraction; antioxidant; DPPH; β-carotene/linoleic acid; Folin–Ciocalteu; antimicrobial; brine shrimp bioassay

Introduction

Today's increasing tendency to use of herbal medicines in the communities and fundamental role of the medicinal plants in the way of drug discovery has caused researchers to be more attracted to this area (1, 2). Caucasian wingnut (*Pterocarya fraxinifolia* L.) from juglandaceae family is one of the Iranian medicinal plants that grows as a tree in the north of Iran, (3). This plant species grows in Euxino-Hyrcanian forests along the southern shores of the Black and Caspian Seas, in Iran, Caucasus and Anatolia (4, 5). At the north of Iran the leaves of this tree have used by indigenous people as a fish poison as well as antifungal agent, dyeing agent, and in the treatment of diarrhoea (6, 7, 8). Fruits and bark of this tree also have used as a diaphoretic agent in Iranian traditional medicine (9). Pervious studies have confirmed antibacterial, antifungal, larvicidal, antioxidant, and toxicity of the leaves of this tree (10, 11). Furthermore, Juglone (an ichthyotoxic principle of the juglandaceae), and two other naphthalene derivatives have been isolated from the leaves and *p*-coumaroylespirmidin derivative from the pollen of Caucasian wingnut during previous phytochemical studies (6, 10, 12) In order to more evaluation of potential medicinal benefits of this tree, the aim of present study is the determination of composition of the essential oils and evaluation of *in vitro* antimicrobial, antioxidant, and cytotoxic activities of the total methanol extracts and volatile fractions of the leaves, bark and fruits of Caucasian wingnut.

Materials and methods

Plant material

The leaves, barks, and fruits of Caucasian wingnut, were collected from four specified trees from a 3 km^2 area of Talesh region (Guilan province, Iran), in September 2011. The voucher specimen of this collection (Voucher No. KBGH 8114) was deposited at the herbarium of Research Institute of Forests and Rangelands, Kashan, Iran.

Volatile fraction extraction

Hundred grams from the air-dried and ground leaves, bark and fruits were individually subjected to simultaneous distillation–extraction (SDE) for 1.5 hours using n-pentane as solvent (13) After evaporation of solvent at room temperature (25°C), residual oil was dehydrated over anhydrous sodium sulphate. Finally obtained pure pale yellowish oils were stored at low temperature (4°C) under nitrogen atmosphere until analysis.

*Corresponding author. Email: m_akhbari@kashanu.ac.ir

GC/mass analysis

GC/MS analysis of the oils was carried out on an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a HP-5MS 5% phenylmethylsiloxane capillary column (30 m × 0.25 mm, 0.25 µm film thickness; Restek, Bellefonte, PA) equipped with an Agilent HP-5973 mass selective detector in the electron impact mode (ionization energy: 70 eV) operating under the same conditions as described above. Identification of components was based on direct comparison of the Kovats indices (K.I.) and computer matching with the Wiley 275.L and Wiley 7n.L libraries as well as by comparisons of the mass spectral data published in the literature (14).

GC/FID analysis

GC analyses were also performed using an Agilent HP-6890 gas chromatograph equipped with a FID detector for quantitative purposes. The FID detector temperature was 290°C. In order to obtain the same elution with GC/MS, same column and same operational conditions were applied. Relative percentages of peak areas were calculated for obtaining quantitative data.

Preparation of extracts

Fifty grams from the air-dried and ground leaves, bark and fruits were individually subjected to extraction using soxhlet apparatus using 500 ml methanol for 8 hours. The extracts were concentrated using a rotary evaporator (BuchiRotavapor R-200, Flawil, Switzerland) under the maximum temperature of 45°C, then dried completely using a vacuum oven (Memmert, VO-400, Germany) working at temperature and pressure of 50°C and 7.5 torr, respectively.

Antioxidant activity

Evaluation of antioxidant activity of the extracts performed using β-carotene/linoleic acid and DPPH free radical-scavenging assays. Total phenolic contents (TPC) of the extracts were also measured using Folin–Ciocalteu test and calculated as gallic acid equivalent (15, 16).

β-Carotene/linoleic acid assay

In this method, antioxidant activity of the extracts was determined based on inhibition of β-carotene oxidation by hydroperoxides arising from linoleic acid described by Miraliakbari and Shahidi (17). Briefly, a stock solution of β-carotene and linoleic acid was prepared with 0.5 mg of β-carotene in 1 ml chloroform, 25 µl of linoleic acid and 200 mg Tween 40. The chloroform was evaporated under vacuum and 100 ml of oxygenated distilled water was then added to the residue. 350 µl from the each extracts sample solution (2 g l^{-1} in DMSO) was added to 2.5 ml of the above mixture in test tubes. The test tubes were incubated in a hot water bath at 50°C for 2 hours. In each assay, Butylatedhydroxytoluene (BHT) was used as a positive control and the same volume of DMSO instead of the extracts as a negative control. The absorbencies were measured at 470 nm wavelength on an ultraviolet spectrometer (Cintra 6, GBC, Australia). Antioxidant activities (inhibition percentage, I%) of the extracts were calculated using the following equation (A; Absorbance):

$$I\% = (A_{\beta\text{-carotene after 2 h assay}}/A_{\text{initial }\beta\text{-carotene}}) \times 100$$

All tests were performed in triplicate and inhibition percentages were reported as means ±SD.

DPPH free radical-scavenging assay

Free radical-scavenging activity of the plant volatile oils and extracts was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging activity assay method described by Sarker et al. (18) with slight modifications. Briefly, stock solutions (10 mg ml^{-1} each) of the oils, extracts, and BHT were prepared in methanol. Serial dilutions were performed to obtain 10 various concentrations from 1 to 9.76×10^{-4} mg ml^{-1}. Diluted solutions (1 ml each) were mixed with 1 ml of a freshly prepared 80 µg ml^{-1} DPPH methanol solution and were kept in the dark at room temperature for 30 min for any reaction to take place. Ultraviolet absorbencies of solutions were measured by spectrometer (Cintra 6, GBC, Australia) at 517 nm. Inhibition of free radical DPPH in percent (I%) was calculated as follow, (A; Absorbance):

$$I\% = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100$$

IC_{50} value, the sample concentration providing 50% inhibition, was calculated for each sample from the graph plotting inhibition percentages against concentrations of the sample. All tests were carried out in triplicate and IC_{50} values were reported as means ±SD.

Total phenolics content assay

TPC of the extracts were measured using Folin–Ciocalteu phenol reagent and gallic acid as a standard (19). The procedure is described briefly, below.

0.1 ml of 100 µg ml^{-1} extract solution was added to 46 ml of a solution containing distilled water and 1 ml of Folin–Ciocalteu phenol reagent. After 3 minutes, 3 ml of 2% Na_2CO_3 solution was added and the mixture was shaken. After two hours, absorbencies were measured at 760 nm. The same procedure was repeated for all the

standard gallic acid solutions (0–100 μg ml^{-1}) and a standard curve was obtained with the following equation:

$$\text{Absorbance} = 0.0012 \times \text{gallic acid } (\mu g) + 0.0033$$

TPC of each extract, was determined as gallic acid equivalent, All tests carried out in triplicate and phenolics content were reported as a gallic acid equivalents as means ±SD.

Antimicrobial activity

Potential antimicrobial activity of the volatile fractions and extracts of Caucasian wingnut were individually evaluated against a set of eleven microorganisms. Following microbial strains were provided by Iranian Research Organization for Science and Technology (IROST): *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Shigelladysenteriae*, *Proteus vulgaris*, *Salmonella paratyphi-A serotype*, *Candida albicans, Aspergillus brasiliensis,* and *Aspergillus niger*. Bacterial strains were cultured overnight at 37°C in nutrient agar (NA) and fungi were cultured overnight at 30°C in sabouraud dextrose agar (SDA).

Disk diffusion assay

Agar disc diffusion method was applied to evaluation antimicrobial effects of the oils and extracts of Caucasian wingnut (20). Plant extract solutions were prepared at the concentration of 30 mg ml^{-1}using DMSO as a solvent and filtered by 0.45 μm Millipore filters for sterilization. 100 μl of suspension containing 10^8 CFU ml^{-1} of bacteria, 10^6 CFU ml^{-1}of yeast and 10^4 spore ml^{-1} of fungi were spread on to the nutrient agar (NA), sabouraud dextrose (SD) agar and potato dextrose (PD) agar mediums, respectively. The discs (6 mm in diameter) impregnated with 10 μl of the volatile oils or the extracts solution (300 μg per disc) and DMSO (as negative control) were placed on the inoculated agar. The inoculated plates were incubated for 24 hours at 37°C for bacterial strains and 48 hours and 72 hours at 30°C for yeast and mold isolates, respectively. Gentamicin (10 μg per disc) and rifampin (5 μg per disc) used for bacteria and nystatin (100 I.U.) were as used for fungi as positive controls. The diameters of inhibition zones were used as a measure of antimicrobial activity and each assay was repeated thrice.

Micro-well dilution assay

Micro-well dilution assay method was used to determination of minimal inhibition concentration (MIC) values of the plant extracts and volatile fractions effective to bacterial strains and yeast in disc diffusion assay (21). The inocula of the microbial strains were prepared from 12 hour broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The extracts and oils dissolved in 10% DMSO and serial twofold dilutions were made in a concentration range of 7.8–500 μg ml^{-1} in 10 ml sterile test tubes containing brain heart infusion (BHI) broth for bacterial strains and sabouraud dextrose (SD) broth for the yeast. Ninety-six-well plates were prepared by dispensing 95 μl of the cultures media and 5 μl of the inoculum into each well. A 100 μl aliquot from the stock solutions of the plant products initially prepared at the concentration of 500 μg ml^{-1} was added into the first well. Then, 100 μl from their serial dilutions were transferred into six consecutive wells. The last well containing 195 μl of the cultures media without the test materials and 5 μl of the inoculum on each strip was used as negative control. The final volume in each well was 200 μl. Gentamicin and rifampin for bacteria and nystatin for yeast were used as standard drugs for positive control in the conditions identical to that of the tests materials. The plates were covered with sterile plate sealers. Contents of each well were mixed on the plate shaker at 300 rpm for 20 seconds and then incubated at appropriate temperature for 24 hours. Microbial growth was determined by the presence of a white pellet on the well bottom and confirmed by plating 5 μl samples from clear wells on NA medium. The MIC value was defined as the lowest concentration of the plant extract required for inhibiting the growth of each micro-organism. All the tests were repeated thrice.

Brine shrimp toxicity assay

A brine shrimp lethality bioassay was carried out to evaluation of the cytotoxic activity of the extracts (22, 23). Brine shrimps (*Artemia salina* leach) were hatched in sterile artificial seawater, prepared by mixing water (2 L), NaCl (46 g), $MgCl_2.6H_2O$ (22 g), Na_2SO_4 (8 g), $CaCl_2.2H_2O$ (2.6 g), and KCl (1.4 g), with a pH of 9.0 adjusted by Na_2CO_3, under constant aeration for 48 hours. After hatching, 10 active nauplii (larvae) were collected by pipette and placed in vials containing 5 ml of prepared artificial seawater solution. Extracts were dissolved in DMSO and diluted with artificial seawater to obtain solutions with 10, 100, 300, 500, 700, and 1000 μg ml^{-1} concentrations. Experiments were conducted by adding extract solutions to the 5 ml of brine solution in a set of three tubes per dose. The vials were maintained at room temperature for 24 hours under light, and then the surviving larvae were counted. The percentage lethality was determined by comparing the mean surviving nauplii of the test and control tubes. The concentration values related to 50% lethality (LC_{50}) were obtained from the best-fit line plotting

Table 1. Chemical composition of the volatile fractions of Caucasian wingnut.

	Compounds[a]	Composition (%)			K.I.[b]	K.I.[c]
		Leaves	Bark	Fruits		
1	2-Hexenal	1.1	–	–	851	855
2	n-Hexanol	1.7	0.2	0.8	861	861
3	2,4-Heptadienal	–	0.2	–	1011	1007
4	M-Pyrol	–	0.1	0.8	1038	1034
5	1-Octanol	–	0.3	–	1070	1068
6	Phenylethyl Alcohol	–	–	2.2	1115	1119
7	1-Menthone	–	1.5	–	1157	1152
8	Menthol	–	3.0	0.6	1174	1171
9	Methyl salicylate	–	-	0.4	1196	1197
10	Menthyl acetate	–	0.4	–	1296	1295
11	Vinyl guajacol	–	0.3	–	1315	1320
12	1,5,5-Trimethyl-6-methylene-cyclohexene	0.8	–	–	1342	1338
13	Eugenol	–	0.3	3.4	1360	1359
14	β-Elemene	–	–	1.7	1395	1393
15	Caryophyllene	12.6	0.3	15.2	1423	1427
16	α-Bergamotene	9.0	–	5.0	1439	1435
17	3¸7-Guaiadiene	2.8	0.6	–	1447	1440
18	α-Humulene	–	0.2	–	1458	1454
19	(E)-β-Farnesene	–	–	3.5	1459	1461
20	Aromadendrene	26.3	–	–	1464	1462
21	Alloaromadendrene	–	0.4	–	1466	1467
22	α-Curcumene	4.2	1.1	5.0	1500	1484
23	β-Selinene	1.6	0.5	14.8	1504	1492
24	Zingiberene	6.6	1.0	1.7	1514	1498
25	β-Bisabolene	0.7	–	–	1520	1512
26	cis-γ-Bisabolene	0.5	–	–	1527	1519
27	β-Sesquiphellandrene	2.3	0.9	1.8	1527	1522
28	(E)-γ-Bisabolene	–	–	0.7	1531	1531
29	Caryophyllene oxide	–	–	10.7	1556	1547
30	Nerolidol	–	1.0	–	1569	1564
31	Palustrol	0.8	–	–	1578	1581
32	ar-Turmerol	–	–	1.5	1583	1583
33	Spathulenol	7.8	–	-	1601	1583
34	Viridiflorol	8.7	–	–	1608	1598
35	Ledol	3.6	–	1.9	1613	1605
36	Humulene epoxide II		1.3	–	1616	1608
37	Biotol	–	37.8	–	1618	1613
38	Eremoligenol	–	–	1.0	1634	1624
39	Cubenol	–	1.1	–	1640	1628
40	Caryophylladienol II	–	–	1.0	1640	1640
41	α-Cadinol	0.8	–	0.6	1646	1645
42	β-Eudesmol	–	1.3	1.3	1654	1650
43	τ-Muurolol	0.2	–	2.1	1654	1650
44	epi- β-Bisabolol	1.1	–	–	1683	1671
45	Khusinol	–	–	2.3	1691	1674
46	Germacra-4(15),5,10,(14)-trien-1-α-ol	–	2.2	1.7	1698	1686
47	Heptadecane	–	0.2	–	1702	1700
48	Cedr-8-en-13-ol	–	–	1.4	1722	1720
49	8(15)-Cedren-9-α-yl-acetate	–	–	1.1	1738	1739
50	Tetradecanoic acid	–	0.8	–	1778	1763
51	Hexahydrofanesyl acetone	–	0.6	–	1848	–
52	Nonadecane	–	0.2	–	1901	1900
53	Palmitic acid	–	2.8	–	1932	1922
54	n-Hexadecanoic acid	–	11.1	3.3	1973	1973
55	Methyl linoleate	–	6.3	–	2103	2089
56	Linolenic acids	–	5.8	-	2111	2098
57	Phytol	–	1.2	–	2120	2110
58	Linolic acid	–	9.1	–	2171	2180
59	Tricosane	–	–	2.0	2297	2300

(*Continued*)

Table 1. (*Continued*)

	Compounds[a]	Composition (%) Leaves	Bark	Fruits	K.I.[b]	K.I.[c]
60	Tetracosane	0.3	–	1.4	2397	2400
61	Pentacosane	0.6	–	2.2	2498	2500
	Oxygenated monoterpens	0.0	4.9	0. 6		
	Hydrocarbon monoterpenes	0.8	0.0	0.0		
	Oxygenated sesquiterpenes	23.1	44.7	26.6		
	Hydrocarbon sesquiterpenes	66.6	5.1	49.3		
	Oxygenated non-terpenes	2.9	37.9	10.8		
	Hydrocarbon non-terpenes	0.9	0.5	5.6		
	Oxygenated diterpenes	–	1.2	–		
	Total identified	94.2	94.3	92.9		

Notes: A dash (–) indicate the absence of compound in the sample. [a]Compounds listed in order of elution from HP-5MS column. [b]Kovats indices to C8–C24 n-alkanes on HP-5MS column. [c]Literature Kovats indices.

concentration vs. percentage lethality. This assay was repeated thrice and lethality percentages were reported as mean ±SD.

Results and discussion

Chemical composition of the volatile fractions

Volatile fraction extraction from the leaves, bark and fruits using simultaneous distillation–extraction (SDE) method yielded 0.20, 0.23, and 0.15% v/w pale yellowish oil, respectively. Twenty-two compounds representing 94.2% of the oil were identified as a result of GC/MS and GC/FID analysis of the leaves volatile fraction (Table 1). The results showed hydrocarbon sesquiterpenes (66.7%) were the major classes of identified compounds and aromadendrene (26.3%), caryophyllene (12.6%) and viridiflorol (8.7%) were the main compounds of the volatile fraction obtained from the leaves (Table 1). Bisabolole oxide A (23.6%) which was reported as a main compound of the essential oil of the leaves of Caucasian wingnut collected from Mazandaran forests (north east of Iran) were not found in our studied sample at all; on the other hand the main compounds of the our studied sample were not reported in the essential oil sample of this plant from Mazandaran (11). To the best of our knowledge, this is the first report of chemical composition of the volatile oil of the bark and fruits of Caucasian wingnut. Thirty-tree and thirty-one compounds, representing 94.3 and 92.9% of the oils were identified in the bark and fruits volatile fraction, respectively (Table 1). These volatile fractions mainly consist of oxygenated sesquiterpenes (44.7% of the bark oil and 26.6% of the fruit oil). Biotol (37.8%) and n-Hexadecanoic acid (11.1%) were the main constituents of the bark oil and caryophyllene (15.2%), β-Selinene (14. 8%) and caryophyllene oxide (10.7%) were identified as the main compounds of the volatile fraction obtained from the fruits of Caucasian wingnut (Table 1).

Antioxidant activity and total phenolic content

Two *in vitro* antioxidant assays, namely 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene/linoleic acid tests were used to evaluate the potential antioxidant activities of the leaves, bark, and fruits of Caucasian wingnut and the results are given in Table 2. In the DPPH assay, leaves and bark extracts showed potent antioxidant activities with IC_{50} values of about 18.19 and 17.9 $\mu g\,ml^{-1}$, respectively, compared with synthetic standard antioxidant BHT ($IC_{50} = \sim 19.7\,\mu g\,ml^{-1}$), whereas the fruits extract showed slight antioxidant activity ($IC_{50} = \sim 79.2\,\mu g\,ml^{-1}$). On the other hand, leaves oil by 43.5% inhibition at the concentration of up to $1\,mg\,ml^{-1}$ showed most free radical scavenging

Table 2. The *in vitro* antioxidant activities of the extracts and volatile fractions (E. oil) of Caucasian wingnut and total phenolics content (TPC) of the extracts.

	Leaf[a]	Bark[b]	Fruit[c]	BHT	Blank
DPPH assay (IC_{50}, $\mu g\,mL^{-1}$)	18.2 ± 0.9	17.9 ± 0.2	79.2 ± 2.6	19.7 ± 0.9	NA
β-Carotene assay (I%)	84.1 ± 0.6	76.3 ± 0.9	83.6 ± 0.8	95.3 ± 0.2	5.3 ± 0.3
TPC[d] ($\mu g\ mg^{-1}$)	137.9 ± 0.9	179.5 ± 1.2	101.4 ± 1.1	–	–

Notes: [a,b,c]Less than 43.5, 19.2 and 17.7% inhibition for the volatile fraction concentrations up to $1\,mg\,ml^{-1}$; [d]Total phenolic content as gallic acid equivalents, NA (not applicable).

activity among the volatile fractions. IC_{50} values reported for the leaves and bark of Caucasian wingnut collected from Mazandaran (~3.89 and 41.57 μg ml^{-1}, respectively) suggests a possible difference in the amounts of antioxidant principles in the plants grow at these two regions (11). Because of the proven role of phenolic compounds as potent free radical scavenger principles in plant extracts, TPC of the extracts were measured using Folin–Ciocalteu test (24, 25). Results of this assay (Table 2) confirm the relative relationship between the amounts of total phenolic content and free radical scavenging activity. Among the extracts, bark extract had highest amount of total phenolic content (~179.5 μg ml^{-1}) with highest free radical scavenging properties ($IC_{50=}$~17.9 μg ml^{-1}) in DPPH assay. The β-carotene/linoleic acid assay is based on inhibition of β –carotene by natural compounds in the presence of linoleic acid hydro peroxide radicals (26). The inhibition of the extracts of the leaves, bark, and fruits on β-carotene oxidation, were 84.1, 76.3 and 83.6%, respectively. These results also confirm considerable capacity of Caucasian wingnut as a valuable natural antioxidant agent (Table 2).

Antimicrobial activity

The antimicrobial activity of the extracts and volatile fractions of the leaves, bark and fruits of Caucasian wingnut were evaluated against a set of eleven microorganisms and their potency were assessed qualitatively and quantitatively by the presence or absence of inhibition zones, zone diameters and MIC values. The results are presented in Table 3 and indicate that extracts and volatile fractions of the plant have considerable antimicrobial activity against some of the gram-positive and gram-negative microorganisms. The extracts of the leaves, bark and fruits showed moderate antimicrobial activities against *S. dysenteriae* in both disc diffusion and micro-well dilution tests. Furthermore, the antimicrobial effects of the bark extract against *S. aureus* and *P. vulgaris* were considerable. Finally, all volatile fractions showed excellent antimicrobial activity against *S. epidermidis* (Table 3). Previous investigation about antimicrobial effects of the leaves extract of Caucasian wingnut has reported its antibacterial activity against *B. subtilis*, antifungal activity against *C. albicans* and *Cladosporium cucumericum* (8). There are several reports relating the antimicrobial activity of natural phenolic compounds (27, 28). According to considerable amount of phenolic content of the extracts of Caucasian wingnut, the observed antimicrobial activities may also be a consequence of the occurrence of such compounds, especially naphthoquinone derivates as bioactive compounds of these extracts. On the other hand based on reports of previous investigations, oxygenated mono and sesquiterpenes can be involved in the appearance of antimicrobial effects of essentials of Caucasian wingnut (29).

Cytotoxic activity

In the brine shrimp lethality assay, high lethal concentrations (LC_{50}) of the leaves, bark, and fruits extracts of Caucasian wingnut were > 1000, 602 ± 5.6 and 400 ± 5.2 μg mL^{-1}, respectively. These results show a very weak cytotoxicity for all the extracts of Caucasian

Table 3. Antimicrobial activity of the extracts and volatile fractions of Caucasian wingnut.

	Samples															
	Leaf				Bark				Fruit				Antibiotics			
	Extract		E. oil		Extract		E. oil		Extract		E. oil		Rif [a]		Gen[b]	
Bacteria	DD[c]	MIC[d]	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC
S. paratyphi-A	12	250	–	–	–	–	–	–	–	–	–	–	–	–	21	500
S. aureus	9	–	10	>500	12	250	9	–	–	–	10	500	10	250	21	500
S. dysenteriae	13	500	–	–	15	500	–	–	12	>500	–	–	8	250	18	500
E. coli	9	–	–	–	10	>500	10	>500	9	–	12	>500	11	500	20	500
K. pneumoniae	–	–	–	–	–	–	–	–	–	–	–	–	7	250	22	250
B. subtilis	–	–	13	250	–	–	12	>500	–	–	11	500	13	15	21	500
P. vulgaris	9	–	–	–	13	500	–	–	9	–	–	–	10	125	23	500
S. epidermidis	10	500	25	250	–	–	29	250	14	125	19	375	40	250	35	500
Yeast and mold													Nys[e]			
													DD		MIC	
C. albicans	–	–	–	–	–	–	–	–	–	–	–	–	33		125	
A. brasiliensis	–	–	11	>500	–	–	9	–	–	–	–	–	23		500	
A. niger	–	250	15	125	–	–	10	>500	–	500	10	>500	27		31	

Notes: A dash (–) indicates no antimicrobial activity. NA; Not applicable.[a]Rifampin, [b]Gentamicin, [c]Inhibition zone in diameter (mm) around the impregnated discs, [d]MICs (as μg mL^{-1}), [e]Nystatin.

wingnut that make it somewhat safe for medical uses. However, more studies on its toxicity properties can be useful to evaluating of the use of Caucasian wingnut as a safe and useful medicinal plant especially for the fruits. This assay was not performable for the volatile oils because of solubility limitations.

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