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Evaluation of antibacterial, antifungal, antiviral, and antioxidant potentials of some edible oils and their fatty acid profiles

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Abstract: In the current study, the oils obtained from the nuts of *Corylus avellana* L. (hazelnut), *Arachis hypogea* L. (peanut, groundnut), *Pinus pinea* L. (umbrella nut), and *Juglans regia* L. (walnut) were tested for their antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and antimicrobial activity against the standard and isolated strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Staphylococcus aureus*, and *Enterococcus faecalis* as well as the fungi *Candida albicans* and *C. parapsilosis* by microdilution method, in which inhibition was expressed as minimum inhibition concentrations (MICs). Moreover, antiviral activity of the oils was determined using *Herpes simplex* (HSV) and *Parainfluenza* (PI-3). Their fatty acid analyses were performed by GC-MS technique. While the hazelnut and walnut oils showed the best antioxidant activity at 100 mg mL^{-1} (73.58% and 65.10%, respectively), the walnut oil had the most potent anti-PI-3 effect (16- < 0.25 μg mL^{-1}). The oils displayed inhibitory potentials towards the isolated strains of *E. coli*, *S. aureus*, and *E. faecalis* at 4, 8, and 8 μg mL^{-1} concentrations, respectively.

Key words: Oil, antibacterial, antifungal, antiviral, antioxidant, fatty acid

Bazı yenilebilen yağların antibakteriyel, antifungal, antiviral ve antioksidan potansiyellerinin değerlendirilmesi ve yağ asitleri profilleri

Özet: Bu çalışmada, *Corylus avellana* L. (fındık), *Arachis hypogea* L. (yerfıstığı), *Pinus pinea* L. (çam fıstığı) ve *Juglans regia* L. (ceviz) meyvalarından elde edilen yağlar, antioksidan aktiviteleri için 2,2-difenil-1-pikrilhidrazil (DPPH) radikaline karşı, antimikrobiyal aktiviteleri için, inhibisyonun minimum inhibitör konsantrasyon (MIK) olarak ifade edildiği mikrodilüsyon yöntemi ile *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Staphylococcus aureus* ve *Enterococcus faecalis*'in standart ve izole suşları ile *Candida albicans* ve *C. parapsilosis* adlı mantarlara karşı test edilmişlerdir. Ayrıca yağların antiviral aktivitesi *Herpes simplex* (HSV) and *Parainfluenza* (PI-3) kullanılarak tayin edilmiştir. Yağ asitleri analizi ise GC-MS tekniği ile gerçekleştirilmiştir. Fındık ve ceviz yağları 100 mg mL^{-1}'de en iyi antioksidan aktiviteyi (sırasıyla % 73,58 ve 65,10) gösterirken, en güçlü anti-PI-3 etkiye de (16-<0,25 μg mL^{-1}) ceviz yağı sahip olmuştur. Yağlar, *E. coli*, *S. aureus* ve *E. faecalis*'in izole suşlarına karşı, sırasıyla, 4, 8 ve 8 μg mL^{-1} konsantrasyonlarda inhibitör potansiyel sergilemişlerdir.

Anahtar sözcükler: Yağ, antibakteriyel, antifungal, antiviral, antioksidan, yağ asiti

Introduction

Oils extracted from plants have been used in many cultures since ancient times and many vegetable oils are consumed directly or used as food ingredients. Vegetable oil consumption throughout the world rose to 87.8 million metric tons (MMT) from 62.6 MMT between 1993 and 2000 (1). Apart from their nutritional properties, nut oils are also used in massage oils, skin care products, and some other cosmetics.

Antioxidants are additives of natural or synthetic origins often used to prevent or discontinue oxidation in foodstuff and cosmetic products. Primary sources of naturally occurring antioxidants are known as whole grains, fruits, and vegetables. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. The main characteristic of an antioxidant is its ability to trap free radicals. A rapid, simple, and inexpensive method to measure antioxidant capacity of food involves the use of the free radical 2.2-diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods. DPPH is a purple-colored free stable radical; when reduced, it becomes the yellow-colored diphenylpicrylhydrazine. The antioxidant activity of various foods can be determined accurately, conveniently, and rapidly using DPPH testing. The trend in antioxidant activity obtained by using the DPPH method is comparable to trends found using other methods reported in the literature.

In the present study, our object was to conduct an antioxidant and antimicrobial screening study on the oils obtained from the nuts of *Corylus avellana* L. (Betulaceae), *Arachis hypogea* L. (Fabaceae), *Pinus pinea* L. (Pinaceae), and *Juglans regia* L. (Juglandaceae). The oils were tested by microdilution method against the following bacteria and fungi: *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Candida albicans*, and *C. parapsilosis*. Their antiviral activities were examined against DNA virus *Herpes simplex* (HSV) and RNA virus *Parainfluenza* (PI-3) on Madin-Darby Bovine Kidney (MDBK) and Vero cell lines. Additionally, antioxidant capacity of the oils through DPPH free radical scavenging was studied. Their fatty acid analyses were performed by GC-MS technique.

Materials and methods

Plant materials

The nuts of *Corylus avellana* L., *Arachis hypogea* L., *Pinus pinea* L., and *Juglans regia* L. were purchased from several stores in Ankara (Turkey) during 2009. The samples are preserved at the Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara (Turkey).

Oil extraction

All samples were powdered and weighed accurately. Then they were independently mixed with anhydrous sodium sulphate and subjected to extraction with *n*-hexane (Merck) for 8 h using a Soxhlet apparatus. The *n*-hexane parts were filtered and concentrated in vacuo. The oil yields (w/w) were as follows: *C. avellana* (43.4%), *A. hypogea* (46.2%), *P. pinea* (53.5%), and *J. regia* (69.1%).

Antimicrobial activity

The oils were dissolved in ethanol:hexane (1:1) by using 51% Tween 80 solution at a final concentration of 1024 μg $mL^{-1}$and sterilized by filtration using 0.22 μm Millipore (MA 01730, USA) and used as stock solutions. Reference antibacterials of ampicillin (AMP, Fako) and ofloxacin (OFX, Hoechst Marion Roussel), as well as reference antifungals of ketoconazole (KET, Bilim) and fluconazole (FLU, Pfizer) were obtained from their respective manufacturers and dissolved in phosphate buffer solution (AMP, pH: 8.0, 0.1 mol L^{-1}), DMSO (KET), in water (FLU, OFX). Stock solutions of the agents were prepared in medium according to the CLSI recommendations. Solvents, pure microorganisms, and pure media were used as control wells. All were tested in triplicate in each run of the experiments (2). Antibacterial activity test was carried out against standard (ATCC; American-type culture collection, RSKK; Culture collection of Refik Saydam Central Hygiene Institute) and isolated (clinical isolate and obtained from Department of Microbiology, Faculty of Medicine, Gazi University) strains.

Standard and the isolated strains of *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 10145), *Proteus mirabilis* (ATCC 7002), *Klebsiella pneumoniae* (RSKK 574), *Acinetobacter baumannii* (RSKK 02026), *Staphylococcus aureus* (ATCC 25923), and *Bacillus subtilis* (ATCC 6633) for antibacterial activity as well as standard strains of *Candida albicans* (ATCC 10231) and *C. parapsilosis* (ATCC 22019) for antifungal activity were employed. Mueller-Hinton Broth (Difco) and Mueller-Hinton Agar (Oxoid) for the bacteria, Sabouraud liquid medium (Oxoid) and Sabouraud dextrose agar (SDA) (Oxoid) for fungi and culture suspensions were prepared using microdilution method as described in our earlier publications (3-5).

Antiviral activity

African green monkey kidney (Vero) and Madin-Darby bovine kidney (MDBK) cell lines as well as the test viruses *Herpes simplex* (HSV) and *Parainfluenza*-3 (PI-3) used in this study were provided by the Department of Virology, Faculty of Veterinary, Ankara University (Turkey). The culture of the cells was grown according to the method we explained previously (4,5). Acyclovir (Biofarma) and oseltamivir (Roche) were used as the reference drugs. Strains of HSV and PIV titers were calculated as $TCID_{50}$ (Tissue Culture Inhibitory Dose) (6). The assay was performed exactly in the same manner as we reported formerly and maximum CPE (cytopathogenic effect) concentrations as the indicator of antiviral activities of the extracts were determined.

Cytotoxicity

MNTC (maximum non-toxic concentration) for each sample was determined by the method described previously (4) based on cellular morphologic alteration.

Antioxidant activity

Antioxidant activity of toluene solutions prepared from the nut oils was tested against DPPH radicals using Ramadan and Moersel's method (7). Briefly, 10^{-4} M DPPH solution prepared in toluene was added to 100 µL of the oil samples prepared at 0.1, 1.0, 10, and 100 µg mL^{-1} concentrations. After each solution was vortexed for 10 s at room temperature, absorbance of the samples at 515 nm against the blank containing only toluene was recorded using a Shimadzu UV-160 A spectrophotometer. Butylatedhydroxy anisol, a synthetic antioxidant, was used as reference.

Derivatization of the oils

According to Morrison and Smith's method (8), the oils independently were weighed in a 50 mL volumetric flask, and then were saponified by adding 12 mL of 0.5 N NaOH prepared in methanol to the mixture and were heated on a steam bath until the fat globules went into solution. Then 20 mL of BF_3/MeOH (Sigma Co.) was added to each flask and the mixtures were boiled for 2 min. After cooling down, the oils were made up to 50 mL with saturated NaCl solution. These mixtures were then transferred to separation funnels independently and extracted with 30 mL of petroleum ether (PE) for each. The PE phases were taken and evaporated on a water bath at 60 °C. Fatty acid profiles were determined as fatty acid methyl esters (FAMEs). The FAMEs were dissolved in *n*-hexane for injection and analyzed by GC-MS. Identification of the peaks was achieved using Wiley databank by comparison of retention times (R_t) and mass spectra of their respective standards. Relative content of % fatty acids was determined with area under peaks using Hewlett Packard software. The results are expressed as an average of 3 determinations in all cases.

Gas chromatography-mass spectrometry (GC-MS) conditions

Chromatographic analysis was carried out on a Hewlett Packard Model 6890/5972 GC system combined with a mass selective detector (GC-MS). The capillary column used was an HP-5MS (5% phenyl methylsiloxane; 30.0 m × 0.25 mm × 0.25 mm, model no: HP 190915-433). Helium was used as carrier gas at a flow rate of 1.0 mL min^{-1}. The samples were analyzed with the column held initially 40 °C after injection with 2 min hold time, then increased to 250 °C with 8 °C min^{-1} heating ramp and final temperature was increased to 250 °C with 1 °C min^{-1} heating ramp with a final hold at 250 °C for 10 min. The injection was performed in split mode (20:1). Both detector and injector temperatures were set at 250 °C. Run time was 30.83 min. MS scan range was (*m/z*): 20-440 atomic mass units (AMU) under electron impact (EI) ionization (70 eV).

Results

Antibacterial and antifungal screening results

According to data obtained from antibacterial and antifungal screening (Table 1), the oils were the most active against ATCC strain of *E. coli* (4 μg mL^{-1}), followed by *S. aureus* and *E. faecalis* (8 μg mL^{-1}). Isolated strains of the bacteria were observed to be more resistant to these oils, which seemed moderately active against standard and isolated strains of *P. mirabilis* and *K. pneumoniae* (16 and 32 μg mL^{-1}, respectively). On the other hand, our oils displayed notable inhibition towards both *C. albicans* and *C. parapsilosis* (8 μg mL^{-1}).

Antiviral and cytotoxicity screening results

As listed in Table 2, HSV was the most resistant virus, and was not affected by any of the oils in this study, exhibiting MNTCs ranging between 8 and 64 μg mL^{-1} on MDBK cells. Interestingly, all of the oils selectively inhibited PI-3 with MNTCs ranging from < 0.25 to 64 μg mL^{-1} on Vero cells. The most effective oil against PI-3 was observed to be the walnut oil, whose maximum and minimum therapeutic gaps were between 16 and < 0.25 μg mL^{-1}, respectively. Considering inhibition of PI-3, the walnut oil was the best, followed by the hazelnut, groundnut, and umbrella nut oils.

Table 1. Antibacterial and antifungal activities of the oils expressed as minimum inhibitory concentrations (MICs) (mg mL^{-1}).

Microorganisms / Oils	*E. coli*		*P. aeruginosa*		*P. mirabilis*		*K. pneumoniae*		*A. baumannii*		*S. aureus*		*E. faecalis*			
	ATCC	Isol.[a]	ATCC	Isol.	ATCC	Isol.	ATCC	Isol.	ATCC	Isol.	ATCC	Isol.	ATCC	Isol.	*C. albicans*	*C. parapsilosis*
C. avellana	4	16	32	64	16	32	16	32	16	64	8	16	8	16	8	8
A. hypogea	4	16	32	64	16	32	16	32	16	64	8	16	8	16	8	8
P. pinea	4	16	32	64	16	32	16	32	16	64	8	16	8	16	8	8
J. regia	4	16	32	64	16	32	16	32	16	64	8	16	8	16	8	8
AMP[b]	2	64	-[g]	-	2	4	2	4	2	4	<0.12	8	0.5	1	NT[h]	NT
OFX[c]	0.12	1	1	4	<0.12	1	<0.12	1	0.12	2	0.5	4	1	2	NT	NT
LVX[d]	<0.12	0.25	1	2	<0.12	1	<0.12	1	0.12	2	0.5	4	0.5	2	NT	NT
KET[e]	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	1	1
FLU[f]	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	4	4

[a]Isol.: Isolated strain, [b]AMP: Ampicillin, [c]OFX: Ofloxacin, [d]LVX: Levofloxacin, [e]KET: Ketoconazole, [f]FLU: Fluconazole, [g]-: No activity observed, [h]NT: Not tested.

Table 2. Antiviral activity and cytotoxicity of the oils against MDBK and Vero cell lines.

	Madin-Darby Bovine Kidney (MDBK) Cells			Vero Cells		
		CPE[b] Inhibitory Concentration			CPE Inhibitory Concentration	
		Herpes simplex			*Parainfluenza*-type 3	
Oils	MNTC[a] (μg mL^{-1})	Maximum	Minimum	MNTC (μg mL^{-1})	Maximum	Minimum
C. avellana	8	-[c]	-	16	8	0.5
A. hypogea	64	-	-	16	8	0.5
P. pinea	64	-	-	64	64	-
J. regia	8	-	-	32	16	<0.25
Acyclovir	16	16	<0.25	-	-	-
Oseltamivir	-	-	-	32	32	<0.25

[a]MNTC: Maximum non-toxic concentration, [b]CPE: Cytopathogenic effect, [c]- : No activity observed

Antioxidant activity results

Antioxidant activity of the nut oils from *C. avellana* (hazelnut), *A. hypogea* (groundnut), *P. pinea* (umbrella nut), and *J. regia* (walnut) was tested against DPPH radical at 0.1, 1.0, 10, and 100 mg mL^{-1} concentrations. The results showed that all of the oils exerted similar scavenging effect towards DPPH at 0.1, 1.0, and 10 mg mL^{-1}, whereas only hazelnut and walnut oils had the best antioxidant activity, displaying 73.58% and 65.10% of inhibition at 100 mg mL^{-1}, respectively (Table 3).

Results of GC-MS analysis of the oils

The oils were examined by GC-MS with respect to their fatty acid contents. Data we obtained revealed that the most prevalent fatty acids detected in the oils of *C. avellana*, *A. hypogea*, *P. pinea*, and *J. regia* were palmitic (10.5%, 11.6%, 9.0%, and 2.7%, respectively), stearic (3.3%, 3.9%, 5.0%, and 5.5%, respectively), oleic (77.4%, 1.4%, 25.7%, and 47.2%, respectively), and linoleic (8.8%, 75.2%, 54.3%, and 25.3%, respectively) acids (Table 4). Among the oils, *A. hypogea* was found to be the richest in linoleic acid quantity (75.2%), followed by *P. pinea* and *J. regia* (54.3% and 25.3%, respectively).

Table 3. Antioxidant activity (Inhibition % ± S.E.M.) of the nut oils against DPPH radical.

	Inhibition % ± S.E.M.[a] against DPPH radical			
Oils	0.1 mg mL^{-1}	1.0 mg mL^{-1}	10 mg mL^{-1}	100 mg mL^{-1}
Coryllus avellana	20.22 ± 1.05	20.69 ± 0.27	31.68 ± 0.05	73.58 ± 1.27
Arachis hypogea	21.06 ± 0.16	22.25 ± 0.84	25.02 ± 0.92	39.55 ± 1.01
Pinus pinea	20.96 ± 0.16	21.82 ± 0.09	25.02 ± 0.92	39.55 ± 1.01
Juglans regia	20.11 ± 0.09	22.01 ± 1.30	31.18 ± 2.26	65.10 ± 0.31
Reference	0.5 mg mL^{-1}	1.0 mg mL^{-1}	2.0 mg mL^{-1}	
Butylated hydroxyanisol	77.99 ± 0.48	81.60 ± 1.67	82.94 ± 0.68	

[a]S.E.M.: Standard error mean (values are mean ± S.E.M. of 3 replicates)

Table 4. Percentage of saturated and unsaturated fatty acids ± S.E.M. in the oils.

Fatty acids	Retention time (min)	*C. avellana*	*A. hypogea*	*P. pinea*	*J. regia*
Saturated					
Palmitic acid (16:0)	11.5	10.5 ± 1.11	11.6 ± 0.84	9.0 ± 0.45	2.7 ± 1.00
Stearic acid (18:0)	13.2	3.3 ± 0.98	3.9 ± 0.97	5.0 ± 0.38	5.5 ± 0.78
Arachidic acid (20:0)	14.6	-[a]	1.7 ± 0.88	-	3.9 ± 0.91
Behenic acid (22:0)	15.8	-	3.3 ± 1.34	0.2 ± 0.13	-
Lignoseric acid (24:0)	17.1	-	1.1 ± 0.65	-	-
Δ9-Saturated					
Palmitoleic acid (16:1, n = 7)	11.2	-	-	0.2 ± 0.53	0.4 ± 0.39
Oleic acid (18:1, n = 9)	13.3	77.4 ± 0.57	1.4 ± 1.23	25.7 ± 0.75	47.2 ± 1.33
Eicosenoic acid (20:1, n = 9)	14.4	-	1.5 ± 0.55	1.4 ± 0.99	2.4 ± 0.86
ω6-Fatty acids					
Linoleic acid (18:2, n = 6)	13.1	8.8 ± 1.20	75.2 ± 1.10	54.3 ± 0.61	25.3 ± 0.71
Total		100.0	99.7	95.8	87.4

[a]- : Not found

Discussion

In our previous study (4), we found a rather noteworthy antiviral effect with pistachio nut oils, which prompted us to perform the current work. In that study, we stated that palmitic acid, having a potent antiviral property against HIV-1 and HIV-2, could be correlated with high antiviral activity of the pistachio nut oils. However, in this case, the oils did not contain palmitic acid in notable amounts, only reaching a maximum of 11.6%. On the other hand, fatty acids as the primary constituents in edible oils have been reported to possess the ability to interfere with bacterial growth or survival (9-14). Antimicrobial activity of fatty acids was stated to be dependant on chain length and unsaturation degree (13,14). Long-chain unsaturated fatty acids exhibit inhibitory activity against many bacteria even including methicillin-resistant *S. aureus* (MRSA) (15). For instance, linoleic and oleic acids were reported as potent antibacterials (16,17). Linoleic acid was also stated as a model compound of unsaturated fatty acids, which selectively inhibits FabI enzyme in *S. aureus* and *E. coli*, catalyzing the final and rate-limiting step of the chain elongation process of the type II fatty acid synthesis (FAS-II) in bacteria (18). Consistently, the oils were dominantly active against *E. coli* (4 μg mL^{-1}), *S. aureus*, and *E. faecalis* (8 μg mL^{-1}). Isaac et al. (10) demonstrated that fatty acid and monoglycerides with 8-12 carbons were more strongly antiviral and antibacterial when added to milk and infant formula than long chain monoglycerides. As given in Table 2, the most active oils towards PI-3 were observed to be *C. avellana* and *J. regia*, 2 oils that contain oleic acid in the highest amounts (77.4% and 47.2%, respectively), which is in conformity with previous data. Nevertheless, sterols are also known to antagonize the antimicrobial activity of fatty acids (19,20). Accordingly, the existence of a variety of sterols, such as β-sitosterol being the most abundant, followed by campesterol, avenosterol, and stigmasterol, was also reported in hazelnut varieties (21). Therefore, lower anti-PI-3 activity of *C. avellana* oil might result from the existence of sterol-type compounds. In a recent study on 3 hazelnut cultivars, oleic acid was found to range between 80.67% and 82.63%, which is similar to our hazelnut oil, and the hazelnut extract was also reported to display strong antibacterial activity against gram-positive bacteria (22). Moreover, our results with the fatty acid analysis of the oils have been found to be compliant with the reported studies (23-25). On the other hand, some studies have been performed to understand the mechanism of antimicrobial action of fatty acids and it was concluded that fatty acids and their esters exhibited non-specific modes of action (26). Against some bacteria, antimicrobial effects of fatty acids were observed to form mostly either by a complete inhibition of oxygen uptake or stimulating uptake of amino acids into the cells and it was suggested that type of inhibition produced by fatty acids is dependant on their concentrations.

Oleic and linoleic acids as well as their derivatives were reported to exert a potent antioxidant effect in different assays (27-31). In addition, tocopherol and derivatives found in walnut were previously found to produce strong antioxidant activity (31-34). Therefore, *trans*-fatty acids and tocopherol derivatives widely found in the oils could be possibly responsible for the antioxidant activity of these oils.

In conclusion, this study underlines that the nuts examined herein are not only sources of energy, but also provide important components, such as monounsaturated and polyunsaturated fatty acids with antioxidant and antimicrobial activities.

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