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Chemical characterization and antimicrobial activity of *Juglans nigra* L. nut and green husk

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Abstract: *Juglans nigra* (Black walnut) is a source of health-supporting biologically active compounds used in traditional medicine. The investigation of bioactive compounds in black walnut could lead to its broader application, as well as to the application of its by-products. Therefore, this study aimed to characterize *J. nigra* nut and green husk based on chemical analysis of their petroleum ether and ethanol extracts obtained by ultrasonic and reflux extraction methods, respectively. Different extract fractions were tested for their antimicrobial activities using Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), Gram-positive bacteria (*Enterococcus faecalis*, *Staphylococcus aureus*) and yeast (reference strain and clinical isolates of *Candida albicans*). The ethanol extracts analysis, performed by high performance liquid chromatography, singled out the ellagic acid as the most dominant compound in nut ($(55.0\pm1.3)\times10^{-3}$ kg m^{-3}) and green husk ($(114.1\pm0.5)\times10^{-3}$ kg m^{-3}) extracts. Non-polar compounds were evaluated using gas chromatography analysis of petroleum ether extracts. *Juglans nigra* nut and green husk contained two saturated fatty acids, palmitic acid (C16:0) and stearic acid (C18:0), then, monounsaturated fatty acids, palmitoleic (C16:1n-7), oleic (C18:1n-9) and vaccenic acid (C18:1n-7), as well as polyunsaturated fatty acids, linoleic (C18:2n-6), γ-linolenic (C18:3n-6) and α-linolenic (C18:3n-3) acids. Ethanol extracts of both *J. nigra* nut and green husk showed antimicrobial activity against *C. albicans*, which is the most common cause of yeast infections.

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INTRODUCTION

Juglans nigra L. (Black walnut) is a deciduous tree whose different parts contain a variety of useful chemical compounds with numerous health benefits.[1–3] The walnut fruit consists of a husk, a hard shell, and kernel. The kernel is eaten raw or roasted, or pressed for oil and contains unsaturated fatty acids and tocopherols that may have beneficial effect on cardiovascular disease risks.[4] The shells are used in a variety of applications ranging from abrasives, fillers, thickeners, and dyes,[5] while the husk is commonly discarded although it contains phenolic compounds that exhibit antioxidant and antimicrobial properties.[4]

The majority of chemical research was focused on the *J. nigra* nuts, which represent a rich source of natural compounds proven to have various medicinal properties.[1,6–8] *J. nigra* nuts are of great biological importance, since they are an excellent source of phytochemical antioxidants, such as a complex mixture of phenolic compounds (phenolic acids, flavonoids and catechins).[3,7,9,10] Nuts of *J. nigra* are not only rich in phenolics, but also contain high levels of phytosterols, unsaturated fatty acids and tocopherols in fatty oil.[10] It is known that the fatty oil from all nuts species is an important parameter in assessing the quality of nuts and responsible for beneficial health effect as well.[6] The fatty acid profile of *J. nigra* kernel consist of unsaturated forms, whereas saturated forms are present in small quantities.[10] Among the unsaturated fatty acids, oleic, linoleic, and *α*-linolenic acids were found in *J. nigra* nuts.[6,10] In addition, *J. nigra* nuts are not only rich in mineral content.[6,11]

Regarding walnut husk, there have been a few studies involving the detailed characterization of phenolic compounds originating from the green husk of common walnut (*J. regia*).[12–14] Generally, the green husk of common walnut is usually discarded in processing, though it contains phenolic compounds that exhibit antioxidant and antimicrobial properties.[13–15] Cosmulescu *et al.*,[12] suggested that the green husk of common walnut as a by-product, is a good raw material for the extraction of phenolics. However, despite a diverse phytochemical composition of *J. regia* green husk,[12–14] there is no detailed chemical analysis of *J. nigra* green husk, except for the study of total phenolics content.[4]

The investigation of bioactive compounds in black walnut could lead to its broader application, as well as its by-products. To date, there is no study that systematically characterizes and compares the chemical profiles between *J. nigra* nut and green husk. Accordingly, the aim of this research was to determine chemical composition and antimicrobial activity of *J. nigra* nut and green husk. The chemical analysis included high performance liquid chromatography (HPLC) and gas chromatography (GC).

EXPERIMENTAL

Plant material

Juglans nigra fruits (nuts with green husks) were collected during September 2021 from one location at Aleksinac in southeast region of Serbia (located at 43º 32' 11"N/, 21º 42′ 11″E). There was not a significant variation among the provenances in number of fruits and fruit weight of different trees from plot. The voucher specimen was deposited at the Herbarium of the Department of Botany, University of Belgrade-Faculty of Pharmacy (HFF), under the number 3906HFF. The fruits were air dried for two weeks at the average temperature of 20 °C in a room with cross ventilation and out of direct sunlight. The green husks were manually removed from the nuts. It was not possible to separate the kernel from the shell, so the whole nut was ground. The nuts and green husks were ground thus obtaining the material particles of average size of 0.75 mm which were stored in a container at −18 °C until use. The extracts of nuts and green husks were made three weeks after collecting the fruits and stored in a container at −18 °C until the use for different analysis (10–20 days).

Extraction process

The 8×10^{-2} kg of powdered nut and green husk of *J. nigra* were extracted with 8×10^{-2} kg of petroleum ether (solvent-to-solid mass ratio 1:1) by indirect ultrasonication using ultrasonic thermostatic bath (Sonic, Niš, Serbia, power 120 W, frequency 40 kHz). Ultrasonic assisted extraction was performed during 80 min at 40 °C, and the obtained suspensions were vacuum filtered. The resulting liquid residues were labeled as the petroleum ether extracts. Each extractives modality was repeated twice.

The 5×10^{-2} kg defatted material of nut and green husk, remaining after the extraction with petroleum ether, was re-extracted with 0.20 dm^3 of 70 % ethanol for 4 h under reflux, at solvent-to-solid mass ratio of 4:1 and at boiling point of the solvent. After the filtration, the resulting liquid residues were labelled as the ethanol extracts. Each extractives modality was repeated twice.

HPLC analysis

The ethanol extracts of nut and green husk were dried under vacuum, dissolved in 70 % ethanol (5 mg cm^{-3}), filtered through 0.45×10^{-6} m filters and analyzed by HPLC using an Agilent LC 1260 system (Agilent Technologies, Waldbronn, Germany) with photodiode-array detector (PDA). Instrumental conditions were as follows: reversed-phase analytical column (Zorbax SB-aq column, 150 m×2.1×10^{-3} m with 3.5×10^{-6} m particle size), 0.35×10^{-6} m^3 min^{-1} flow rate, 25 °C temperature, 2×10^{-9} m^3 injection volume. Solvent A, 0.1% formic acid in water , and solvent B, acetonitrile, were used for gradient elution: initial 10 % of B, rising to 30 % in 35 min, 35–45 min rising to 70 % of B and returning to initial conditions till 55 min. The wavelengths on which chromatograms were recorded were 210, 250, 320 and 350 nm. Qualitative analysis was performed by comparing the UV spectrum and the retention time of the detected component with those obtained for ellagic acid (Sigma–Aldrich). For the quantification of ellagic acid external standard method was used, with standard of ellagic acid at 250 nm (y = 54x–209, R^2 = 0.9993, working concentration range 17×10^{-3}–150×10^{-3} kg m^{-3}; *LoD* 6.3×10^{-3} kg m^{-3}, *LoQ* 19×10^{-3} kg m^{-3}, calculated according to ICH using residual standard deviation of calibration curve). HPLC analyses was performed in duplicate for each extractives modality.

GC analysis

The fatty acids composition of petroleum ether extracts of nut and green husk was determined using the method by Glaser *et al*., with few modifications.[16] Briefly, in 100×10^{-9} m^3 of petroleum ether extracts 1.5×10^{-6} cm^3 of 3 M HCl in methanol was added and heated for 60 min at 85 °C. After cooling to room temperature, 1.5×10^{-6} m^3 of hexane was added and centrifuged for 15 min at 3000 rpm. The hexane layer was evaporated to dryness and stored in the freezer until analysis. Fatty acid methyl esters were analyzed by gas chromatography using a Shimadzu gas chromatograph with a flame ionization detector (model 2014) equipped with an Rtx 2330 column (60 m, 0.25×10^{-3} m inside diameter, film thickness 0.25×10^{-6} m, Restek, USA). Helium was used as the carrier gas with a flow rate of 1 mL/min. The temperature of the injector was 220 °C, of detector 260 °C, while the initial oven temperature of 130 °C was held for 10 min and then programmed to increase 3 °C min^{-1} to a final oven temperature of 220 °C. The individual fatty acids were identified using fatty acids standard mixtures PUFA-2 and Supelco 37 Component FAME Mix (Sigma–Aldrich). The results for individual fatty acids were expressed as the percentage of total identified fatty acids (in mol %). GC analyses was performed in triplicate for each extractives modality.

Antimicrobial activity

The antimicrobial effect was examined against the following microorganisms: reference strains of Gram-negative bacteria *Escherichia coli* ATCC 25922 (Kwik-stik, Microbiologics Inc.), *Pseudomonas aeruginosa* ATCC 27853 (Kwik-stik, Microbiologics Inc.) and Gram-positive bacteria *Enterococcus faecalis* ATCC 29212 (Kwik-stik, Microbiologics Inc.), *Staphylococcus aureus* ATCC 25923 (Kwik-stik, Microbiologics Inc), as well as the reference strain of *Candida albicans* ATCC 10231 (Kwik-stik, Microbiologics Inc.) and two clinical isolates of *C. albicans*. Gram-negative bacteria can cause serious diseases in humans, especially in immuno-compromised individuals. The cell wall of Gram-negative bacteria is a more extensive and rigid complex than that of Gram-positive species, which is why Gram-negative bacteria species are more resistant. The clinical oral isolates of *C. albicans* were collected from patients diagnosed with prosthetic stomatitis, from the Department of Prosthodontics, School of Dental Medicine, University of Belgrade (Serbia). From the surface of the denture base, a biofilm was obtained using a method of denture sonication described previously.[17] The obtained solution was homogenized and 5×10^{-8} m^3 was plated on CHROM agar *Candida* medium (CHROMagar, Paris, France) and incubated at 37 °C for 48 h. The identification of *Candida* strains was done according to the specific appearance of colonies as defined by the manufacturer.

The antimicrobial activity of petroleum ether and ethanol extracts was assessed using broth microdilution method according to EUCAST.[18] The yeast and bacterial suspensions were prepared in sterile saline and adjusted to 0.5 McFarland standard of turbidity (which corresponds the size of inoculums of 1×10^{12}–5×10^{12} CFU m^{-3}). When testing the bacterial susceptibility, volume of 9×10^{-8} m^3 of dextrose broth (Himedia) and volume of 1×10^{-8} m^3 of each bacterial suspension was added to the wells of a sterile 96-well microtiter plate containing volume of 1×10^{-7} cm^3 of nut and green husk extracts diluted in dextrose broth. When testing yeast susceptibility, volume of 9×10^{-8} m^3 of RPMI 1640 medium (Merck) and volume of 1×10^{-8} m^3 of each fungal suspension was added to the wells of a sterile 96-well microtiter plate already containing volume of 1×10^{-7} m^3 of extract in RPMI 1640 medium. The microplates were then incubated for 24 h at 37 °C. The serial dilutions of nut and green husk extracts were made in the microtiter plate wells. The nut and green husk extracts concentrations of 3.38, 2.75, 1.69 and 1.38 kg m^{-3} were tested.

The minimal inhibitory concentration (*MIC*) was the lowest concentration where microorganism growth was not observed after 24 h. The test for antifungal activity also included positive control (fungi in RPMI 1640 without ethanol and petroleum ether fraction) and negative control (only RPMI 1640 without yeast suspensions). Positive controls were represented with wells containing a bacterial suspension in an appropriate growth medium, as well as a bacterial suspension in an appropriate growth medium with ethanol or petroleum ether in concentration corresponding to the highest used in the broth microdilution assay. In parallel with the examination of the effect of extracts in a given solvent, the effect of the solvent itself (70 % ethanol, petroleum ether) was examined as well. Negative controls were represented with wells containing growth medium and plant extract. All measurements of MIC values were repeated in triplicate.

RESULTS AND DISCUSSION

The total extractive substances of petroleum ether and ethanol extracts

The concentration of total extractive substances in petroleum ether extracts was 0.2±0.02 and 0.25±0.02 kg m^{-3} for nut and green husk, respectively. The concentration of total extractive substances in ethanol extracts was 11.0±1.4 and 13.5±1.5 kg m^{-3} for nut and green husk, respectively.

HPLC analysis of ethanol extracts

The ethanol extracts of *J. nigra* nut and green husk were analyzed by HPLC--PDA. In the both extracts, ellagic acid was the most dominant compound (Fig. 1). The concentration of ellagic acid in ethanol extracts of nut and green husk was (55.0±1.3)×10^{-3} and (114.1±0.5)×10^{-3} kg m^{-3} of extracts, respectively.

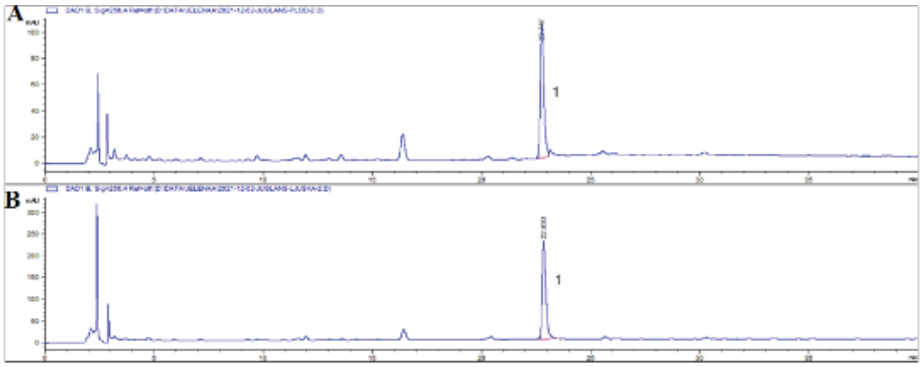

Fig. 1. HPLC chromatogram of *J. nigra* nut (A) and green husk (B) ethanol extracts recordered at 250 nm (1-ellagic acid).

This study showed that *J. nigra* nut is rich in ellagic acid, which is in accordance with previous research.[9] Phenolic profiles of previously reported *J. nigra* kernels[9,10] to some extent differ from phenolic profile of *J. nigra* nut analyzed in this study, probably due to different growth conditions, as well as to the different starting materials and conditions of extraction.[9,10]

Although there is information in the literature regarding *J. nigra* kernel phenolic profile,[8–10] data are missing for green husk. So far, for *J. nigra* green husk the high total phenolic content has been determined by Wenzel and co--workers.[4] Our comparative analysis showed that green husk is generally richer in ellagic acid than nut. Ellagic acid is detected at high concentrations in many berries (strawberries, raspberries, cranberries and grapes), and other known sources of ellagic acid, include walnuts;[19] however, this study points out that *J. nigra* green husk represent a rich source of ellagic acid, as well.

Ellagic acid is a naturally occurring polyphenolic lactone component of some fruits and vegetables.[19,20] It is well known for its antioxidant activity[21] and also possess various pharmacological activities including anti-inflammatory, hepatoprotective, neuroprotective[22] and antiatherogenic,[23] suggesting its potential beneficial health effects.

GC analysis of petroleum ether extracts

Fatty acid compositions were analyzed for petroleum ether extracts of *J. nigra* nut and green husk. Table I lists the fatty acids of two petroleum ether extracts. *J. nigra* nut and green husk containing two saturated fatty acids (SFA), palmitic acid (C16:0) and stearic acid (C18:0), then monounsaturated fatty acids (MUFA) palmitoleic (C16:1n-7), oleic (C18:1n-9), and vaccenic acid (C18:1n-7), as well as polyunsaturated fatty acids (PUFA) linoleic (C18:2n-6), γ-linolenic (C18:3n-6) and α-linolenic (C18:3n-3) acids.

TABLE I. Fatty acid profile of petroleum ether extracts of *J. nigra* nut and green husk. The results for individual fatty acids were expressed as the percentage of total identified fatty acids (in mol %); SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids

Fatty acid		Plant part	
		Nut	Green husk
		SFA	
C16:0	Palmitic acid	8.06±0.29	12.45±0.09
C18:0	Stearic acid	3.56±0,19	3.39±0.03
Total SFA		11.66±0.48	15.84±0.12
		MUFA	
C16:1(n-7)	Palmitoleic acid	0.23±0.10	0.51±0.03
C18:1(n-7)	Vaccenic acid	2.75±0.22	2.96±0.94
C18:1(n-9)	Oleic acid	41.34±0.31	31.08±1.05
Total MUFA		44.09±0.61	34.04±2.2
		PUFA	
C18:2(n-6)	Linoleic acid	36.35±0.57	45.15±0.03
C18:3(n-6)	γ-Linolenic acid	0.54±0.09	0.50±0.11
Total n-6 PUFA		36.89±0.66	45.65±0.14
C18:3(n-3)	α-Linolenic acid	7.06±0.19	3.96±0.05
Total PUFA		43.95±0.87	49.61±0.19

Among the saturated fatty acids, the concentration of palmitic acid detected in nut was lower than in green husk, while the concentration of stearic acid was similar in nut and green husk of black walnut (Table I). Similar levels of palmitic (7.03 %) and stearic acids (2.75 %) were reported for *J. regia* nut.[24] On the other hand, the percentage of oleic acid in *J. nigra* nut (41.34 %) was significantly higher compared to *J. regia* (14.47 %),[22] making black walnut a rich source of oleic acid.[6] Although the green husk is also rich in MUFA, significantly higher levels were found in the nut (Table I). Oleic acid demonstrates large spectra of biological activities associated with many beneficial health effects. On the cellular level, as a part of membrane phospholipids, oleic acid increases membrane fluidity and transport, stimulates enzymatic activity, and regulates the activity of membrane receptors and signal transduction and transcription of some genes.[25] Numerous epidemiological observations have showed that practicing the Mediterranean diet, rich in oleic acid and nuts, is associated with the decrease of the total cholesterol, low-density lipoprotein cholesterol and triglyceride concentrations in plasma, having thus positive effect on cardiovascular and coronary artery disease.[26] Moreover, it has been showed that Mediterranean diet is positively associated with the bone health, a reduced incidence of cancer, Parkinson's and Alzheimer's diseases, and all-cause mortality.[24,27]

In both *J. nigra* nut and green husk, unsaturated fatty acids content was very high, 84.16 and 88.26 %, respectively. In addition to oleic acid, the nut and particularly green husk are valuable sources of linoleic acid (LA, Table I). In comparison to other walnuts such as *J. regia* (63.15 %),[24] LA was found in considerably lower amounts in *J. nigra* (36.35 %). LA is an essential fatty acid that is indispensable for normal growth and development, and its recommended daily intake is around 10 g day^{-1}. As it can be seen in Table I, α-linolenic (ALA) content of *J. nigra* nuts is significant, 7.06 %. The richest sources of ALA are flaxseed oil (53 %), walnuts (11 %) and canola oil (7 %) as well as rosehip seed oil (about 20 %).[28,29] Accordingly, the consumption of *J. nigra* nuts may have favourable effects on general health.

Other unsaturated fatty acids were also detected: palmitoleic acid, vaccenic acid and γ-linoleic acid, which have a low content (<3 % of total fatty acid). When comparing fatty acids profile of *J. nigra* nut and green husk, it can be concluded that nuts have more favourable composition, with higher levels of oleic acid and ALA, and lower levels of SFA and n-6 PUFA.

Antimicrobial activity petroleum ether and ethanol extracts

The antimicrobial activity of different *J. nigra* nut and green husk extracts was assessed against a diverse range of microorganisms, including both Gram-positive and Gram-negative bacteria, and a yeast *C. albicans*. The ethanol extracts of nut and husk didn't show inhibitory activity against the Gram-negative

and Gram-positive bacteria, whereas petroleum ether extracts of nut and husk didn't show inhibitory activity against any of the tested microorganisms (Table II). Moreover, the ethanol extracts of nut and green husk displayed the ability to inhibit *C. albicans* growth (Table II). The ethanol extract of nut showed *MIC* of 1.38±0.01 kg m^{-3}, while the ethanol extract of green husk showed MIC of 1.69±0.04 kg m^{-3} against reference strain and clinical oral isolate *C. albicans*.

TABLE II. Antimicrobial activity of the extracts of nut and green husk from *J. nigra*

Microorganism	Petroleum ether extract		Ethanol extract	
	Nut	Green husk	Nut	Green husk
Gram-positive bacteria				
S. aureus	–	–	–	–
E. faecalis	–	–	–	–
Gram-negative bacteria				
E. coli	–	–	–	–
P aeruginosa	–	–	–	–
Yeast				
C. albicans	–	–	+	+

The presence of the phenolic compounds in ethanol extracts of nut and husk hints at possible inhibitory effects on *C. albicans* growth. Phenolics, such as gallic acid, ellagic acid, ferulic acid and naringin, have been linked to antimicrobial activity.[10] Recently, the ellagic acid have been reported to show inhibitory effects on *C. albicans*,[20] so ellagic acid present in the ethanol extracts of *J. nigra* could be related to the demonstrated anticandidal activity. Interestingly, the study revealed the contributions of ethanol extracts from nut or green husk to the antifungal activity.

This research showed that the ethanolic extract of *J. nigra* nut and husk exhibits higher anticandidal activity as compared to methanolic extracts of *J. regia*.[30] On the other hand, the ethanol extract of *J. nigra* nut and husk showed less inhibitory effect against the tested oral Candida strains than the ethyl acetate extract of *J. regia* bark.[31] The differences in the *MIC* values obtained in our and previous studied can be explained by different chemical composition of various *Juglans* species and their parts used for antifungal examination.

The ellagic acid has been identified in ethanol extracts of *J. nigra*. The presence of the phenolic compounds in ethanol extracts of nut and husk hints at possible inhibitory effects on *C. albicans* growth. Phenolics, such as gallic acid, ellagic acid, ferulic acid and naringin, have been linked to antimicrobal activity.[10] Recently, the ellagic acid have been reported to show inhibitory effects on *C. albicans*,[32] so ellagic acid present in the ethanol extracts of *J. nigra* could be related to the demonstrated anticandidal activity. A study regarding the mechanism of antifungal activity of ellagic acid showed that this compound inhi-

bits biosynthesis of ergosterol and reduces the activity of membrane sterols.[33] Ergosterol (sterol) is an important component of the fungal membrane responsible for maintaining its stability and fluidity, enzyme activity and transport processes.[34] The inhibitory activity of *J. nigra* nuts and green husk ethanolic extracts originates from the presence of ellagic acid, which contributes to the dysfunction of the fungal membrane. The significance of this study is elucidating the impact of ethanolic extracts from walnut or green husk on the inhibition of clinical oral isolates of *C. albicans*, thus paving the way to the possible use of these extracts in antimicrobial treatment in oral hygiene.

CONCLUSION

The petroleum ether and ethanol extracts of *J. nigra* nut and green husk were chemically analysed and their antimicrobial activity was examined. Ellagic acid was found to be the most dominant phenolic compound in the ethanol extracts of *J. nigra* nut and green husk. Fatty acids analysis identified saturated fatty acids (palmitic acid and stearic acid), monounsaturated fatty acids (palmitoleic, oleic, and vaccenic acid) and polyunsaturated fatty acids (linoleic, γ-linolenic, and α--linolenic acids) in petroleum ether of *J. nigra* nut and green husk. None of the extracts had antimicrobial potency for Gram-positive and Gram-negative bacteria. The petroleum ether extracts didn't show the ability to inhibit *C. albicans* growth, while the ethanol extracts of *J. nigra* nut and green husk showed the ability to inhibit *C. albicans* growth. The obtained results support the possible use of these extracts in the antimicrobial treatment in oral hygiene. In conclusion, the results proved that nut and the green husk of *J. nigra*, considered as by-products, can actually be the raw material for the extraction of components potentially beneficial to human health.

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ИЗВОД

ХЕМИЈСКА КАРАКТЕРИЗАЦИЈА И АНТИМИКРОБНА АКТИВНОСТ ОРАШАСТОГ ПЛОДА И ЗЕЛЕНЕ ЉУСКЕ *Juglans nigra* L.

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Juglans nigra (црни орах) као извор биолошки активних једињења се користи у традиционалној медицини. Истраживање биоактивних једињења присутних у црном ораху може довести до шире примене његових производа. Зато ова студија има за циљ да окарактерише орашаст плод и зелену љуску J. nigra на основу хемијске анализе њихових петролетарских и етанолних екстраката, добијених ултразвучним и рефлукс методама екстракције. Антимикробна активност различитих фракција екстракта је тестирана коришћењем Грам-негативних бактерија (*Escherichia coli*, *Pseudomonas aeruginosa*), Грам-позитивних бактерија (*Enterococcus faecalis*, *Staphylococcus aureus*) и квасница (референтни сој и клинички изолати *Candida albicans*). Анализа течном хроматографијом високих перформанси, издвојила је елагинску киселину као најдоминантније једињење у етанолним екстрактима орашастог плода ($(55{,}0\pm1{,}3)\times10^{-3}$ kg m^{-3}) и зелене љуске ($(114{,}1\pm0{,}5)\times10^{-3}$ g m^{-3}). Неполарна једињења су одређена применом гасне хроматографске анализе петролетарских екстракта. Орашаст плод и зелена љуска садрже две засићене масне киселине, палмитинску (C16:0) и стеаринску киселину (C18:0), затим мононезасићене масне киселине, палмитолеинску (C16:1n-7), олеинску (C18:1n-9) и вакценску киселину (C18:1n-7), као и полинезасићене масне киселине, линолну (C18:2n-6), γ-линоленску (C18:3n-6) и α-линоленску (C18: 3n-3) киселину. Етанолни екстракти орашастог плода и зелене љуске показали су антимикробну активност на сојевима *C. albicans* која је најчешћи узрочник гљивичних инфекција.

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