Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 29 July 2026
Revised 23 Aug 2026
Accepted 07 Sep 2026

Natural Resources for Human Health 2026, Vol. 6 (21s): 1415–1422

KEYWORDS:

Aesculus hippocastanum, seed oil, fatty acid composition, GC-FID, antimicrobial activity, agar diffusion.

Fatty Acid Composition of Aesculus Hippocastanum Seed Oil and its Antimicrobial Activity

Shahnoza Isakova[1*], Vazira Nurmukhamedova[2], Rakhimov Dilshod[3], Abbos Nurmukhamedov[4], Munisa Nurullayeva[5], Veronika Abduazizova[6], Farahmon Nasriyev[7]

[1,2,3,4,5,7]Tashkent Institute of Chemical-Technology, Tashkent, Uzbekistan;
[6]Namangan State Technical University, Namangan, Uzbekistan;
Corresponding author: Shahnoza Isakova email:sahnozai220@gmail.com

ABSTRACT:

Aims:
Aesculus hippocastanum L. (horse chestnut) seed oil is a promising source of bioactive compounds; however, its chemical composition in Uzbekistan remains poorly characterized. This study aimed to determine the fatty acid profile of seed oil and evaluate its antimicrobial activity against selected pathogenic microorganisms.

Methodology and results:
Seeds oil were extracted using hexane and analyzed by gas chromatography with flame ionization detection (GC-FID). Antimicrobial activity was assessed using the agar well diffusion method against representative bacterial and fungal strains. The oil yield was 6.5% (w/w). Fatty acid analysis revealed a predominance of unsaturated fatty acids (89.7%), mainly oleic acid (51.46%) and linoleic acid (30.82%), while saturated fatty acids accounted for 10.3%. The oil demonstrated antimicrobial activity against Staphylococcus aureus (19.87 ± 0.15 mm), Klebsiella oxytoca (16.80 ± 0.26), Proteus mirabilis (15.00 ± 0.10), Pseudomonas aeruginosa (13.93 ± 0.35), and Candida albicans (11.50 ± 0.50). No inhibitory effect was observed against Escherichia coli and Bacillus subtilis.

Conclusion, significance and impact of study:
The results indicate that A. hippocastanum seed oil from Uzbekistan is rich in unsaturated fatty acids and exhibits selective antimicrobial activity. These findings highlight its potential application as a natural source of bioactive compounds for nutraceutical and antimicrobial formulations, supporting its further investigation in food and pharmaceutical industries.

INTRODUCTION

Plant-derived natural products remain an important source of bioactive compounds for pharmaceuticals, functional foods, and nutraceuticals. In recent years, increasing attention has been directed toward plant oils rich in unsaturated fatty acids because of their reported roles in cardiovascular health, immune regulation, anti-inflammatory activity, and antimicrobial effects. Natural products continue to contribute substantially to drug discovery and remain widely used in both traditional and modern medicine (**Newman and Cragg, 2020; Sacks et al., 2017; Naghshi et al., 2021**).

Aesculus hippocastanum L. (horse chestnut) is a well-known medicinal plant whose seeds contain triterpene saponins, flavonoids, tannins, and fixed oils. Horse chestnut seed preparations have been extensively investigated for their venotonic, vascular-protective, and anti-inflammatory properties and have shown clinical benefits in the management of chronic venous

insufficiency **(Baraldi et al., 2007; Sirtori, 2001; Pittler and Ernst, 1998).** In addition to their pharmacological importance, plant-derived oils rich in unsaturated fatty acids have demonstrated antimicrobial activity against a wide range of pathogenic microorganisms, highlighting their potential as natural alternatives to synthetic preservatives and antimicrobial agents **(Štumpf et al., 2023; Dahash et al., 2021; Dridi et al., 2023)**. Although the chemical composition and biological properties of A. hippocastanum have been investigated in several European and Asian countries, information on the fatty acid composition and antimicrobial activity of seed oil from Central Asian populations remains limited. Environmental factors, including genotype, climate, and soil conditions, may influence lipid biosynthesis and consequently affect the fatty acid profile and biological activity of the oil **(Čukanović et al., 2020; Zlatanov et al., 2013)**. Furthermore, most previous studies have focused on aqueous or ethanolic extracts, whereas information regarding the antimicrobial potential of the fixed seed oil is still scarce **(Štumpf et al., 2023; Dridi et al., 2023)**. Therefore, the present study aimed to determine the fatty acid composition of A. hippocastanum seed oil collected in the Tashkent region of Uzbekistan using GC-FID and to evaluate its antimicrobial activity against selected Gram-positive, Gram-negative, and fungal microorganisms using the agar well diffusion method **(Balouiri et al., 2016)**.

MATERIALS AND METHODS

Preparation of seeds

Mature seeds of *Aesculus hippocastanum* L. were collected in October from trees growing in the Qibray district, Tashkent region, Republic of Uzbekistan (altitude 450 m, typical continental climate with hot summers and cold winters). Seeds were air-dried at room temperature (20–25 °C) for 2 weeks, manually dehulled to remove seed coats, and ground to a particle size of approximately 3 mm using a laboratory mill. The ground material was stored in airtight containers at 4 °C until extraction. The storage period did not exceed four weeks prior to extraction.

Oil extraction

Oil was extracted from 600 g of ground seeds using n-hexane (analytical grade) in a solid-to-solvent ratio of 1:5 (w/w). Extraction was performed at room temperature (22–25 °C) by maceration with periodic shaking (five successive extractions, each for 12 h). The combined extracts were filtered through filter paper, and the solvent was evaporated under reduced pressure at 40 °C using a rotary evaporator (Heidolph, Germany). The obtained oil was weighed, and the yield was calculated as percentage (w/w) relative to the dry seed weight. The crude oil was stored at 4 °C in dark glass vials under nitrogen until further analysis **(Amiri et al.,2019).**

Preparation of fatty acid methyl esters (FAMEs)

Fatty acids in the seed oil were converted to methyl esters following a saponification–methylation procedure adapted from standard methods. Briefly, 100 mg of oil was saponified with 10% methanolic KOH (1:10, v/v) by heating in a water bath at 80°C for 1 h. The hydrolysate was neutralized with 50% aqueous H_2SO_4 to pH = 2. Free fatty acids were extracted three times with diethyl ether. The combined ether extracts were washed with distilled water until neutral (pH 6.5–7.0), dried over anhydrous Na_2SO_4, and the solvent was evaporated under a gentle nitrogen stream.

The resulting total fatty acid fraction was methylated using ethereal diazomethane (generated from N-methyl-N-nitroso-p-toluenesulfonamide) at room temperature (20-25°C) until a persistent yellow color indicated excess reagent. Excess diazomethane was removed by gentle heating. The FAME mixture was purified by thin-layer chromatography (TLC) on silica gel 60 plates (Merck, Germany) using hexane:diethyl ether (4:1, v/v) as the mobile phase. Plates were visualized under iodine vapor, and the FAME band (Rf = 0.6–0.7) was scraped off and desorbed with chloroform. After solvent evaporation, purified FAMEs were redissolved in n-hexane for GC analysis.

Gas chromatography analysis

FAMEs were analyzed on an Agilent 6890N gas chromatograph (Agilent Technologies, USA) equipped with a flame ionization detector (FID) and an HP-5 capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness; 5% phenyl–95% methylpolysiloxane stationary phase). Operating conditions were as follows: injector temperature 250 °C (splitless mode), detector temperature 280 °C, helium carrier gas at constant flow (1.0 mL/min). The oven temperature program started at 150 °C (held 2 min), ramped at 4 °C/min to 270 °C (held 10 min). Injection volume was 1 μL. Fatty acids were identified by

comparing retention times with those of authentic FAME standards (Supelco 37 Component FAME Mix or equivalent) and quantified by area normalization **(Christie, W.W.,1993).** Results are expressed as relative percentage (%) of total identified fatty acids.

Antimicrobial activity assay

Antimicrobial activity of the seed oil was evaluated using the agar well diffusion method **(Balouiri et al., 2016).** Test microorganisms included the following conditionally pathogenic strains: *Escherichia coli* NC 101, *Pseudomonas aeruginosa* 003841/114, *Proteus mirabilis* 6, *Bacillus subtilis* VKM, *Klebsiella oxytoca* 1, *Staphylococcus aureus* D2, and *Candida albicans* (obtained from the Laboratory of Microbiology, Tashkent Institute of Chemical Technology, Tashkent, Uzbekistan).

Previously prepared Mueller–Hinton agar (HiMedia, India) according to CLSI guidelines (**CLSI 2022**) was poured into Petri dishes (20 mL per plate). Overnight cultures (18–24 h) were suspended in sterile 0.9% NaCl to achieve a turbidity equivalent to 0.5 McFarland standard (=1–2 × 10^7 CFU/mL). The suspension was evenly spread on the agar surface using a sterile cotton swab. Wells of 6 mm diameter were punched in the agar with a sterile cork borer, and each well was filled with 100 µL of undiluted seed oil using a micropipette.

Plates were pre-diffused at 4 °C for 24 h, then incubated at 36 ± 1 °C for 18 h. Inhibition zone diameters (including the well) were measured in millimeters using a caliper. All tests were performed in triplicate. A negative control (sterile hexane) was included during the assay. Activity was classified as no activity (0 mm), weak (≤12 mm), moderate (13–17 mm), or strong (≥18 mm) based on inhibition zone diameter **(Lalpuria et al., 2013).**

Statistical analysis

Data are presented as mean ± standard deviation (SD) of three independent measurements (n = 3). Differences among inhibition zone diameters of susceptible microorganisms were analyzed using one-way analysis of variance (ANOVA). Statistical significance was accepted at $p < 0.05$. Statistical analyses were performed using GraphPad Prism 8.0.

RESULTS AND DISCUSSION

Oil yield and fatty acid composition

Hexane extraction of dehulled *Aesculus hippocastanum* L. seeds collected from the Qibray district (Tashkent region, Uzbekistan) yielded 6.5% (w/w) crude oil (39 g from 600 g dry ground seeds). This yield aligns with the reported range of 4.13–8.0% for horse chestnut seeds across different geographical origins **(Zlatanov et al., 2013; Čukanović et al., 2020).**

The fatty acid profile, analyzed by GC-FID of fatty acid methyl esters (FAMEs), revealed a composition dominated by unsaturated fatty acids (89.7%), with saturated fatty acids accounting for only 10.3% (Table 1). The major fatty acids were oleic acid (C18:1 n-9, 51.46%), linoleic acid (C18:2 n-6, 30.82%), palmitic acid (C16:0, 7.27%), eicosenoic acid (C20:1 n-9, 5.75%), and stearic acid (C18:0, 2.47%). Linolenic acid (C18:3 n-3) was present at 1.52%, with minor contributions from myristic, margaric, arachidic acids and traces of capric and lauric acids.

This profile is broadly consistent with previous studies on *A. hippocastanum* seed oils from Europe and Asia, where unsaturated fatty acids typically comprise 87–92%, with oleic acid as the predominant component and linoleic acid ranging from 16–30% **(Baraldi et al., 2007; Zlatanov et al., 2013; Čukanović et al., 2020).** European samples have shown oleic acid dominance up to 65% with lower linoleic acid content (20–21%), whereas variation in lipid composition is strongly influenced by genotype and environmental conditions **(Čukanović et al., 2020; Sirtori, 2001).** The higher linoleic acid content in the present Uzbek sample may reflect climatic and soil-related factors influencing lipid biosynthesis **(Čukanović et al., 2020; Zlatanov et al., 2013).**

The high proportion of monounsaturated (oleic + eicosenoic = 57.2%) and polyunsaturated (linoleic + linolenic = 32.3%) fatty acids underscores the oil's potential nutritional value. Oleic acid contributes to hypocholesterolemic and anti-inflammatory effects, while linoleic and α-linolenic acids serve as essential precursors for eicosanoids, supporting cardiovascular health, membrane integrity, and immune function. The low saturated fat content further enhances its suitability as a dietary lipid source for cholesterol management **(Sacks et al., 2017; Naghshi et al., 2021).**

Natural Resources for Human Health
eISSN: 2583-1194

Table 1. Fatty acid composition of *Aesculus hippocastanum* L. seed oil

No.	Fatty acid	Fatty acid index		Content, % mean ± SD (n = 3)
1	Capric acid	C10:0		trace
2	Lauric acid	C12:0		trace
3	Myristic acid	C14:0		0.12 ± 0.01
4	Pentadecanoic acid	C15:0		-
5	Palmitic acid	C16:0		7.27 ± 0.05
6	Palmitoleic acid	C16:1	n-7	0.15 ± 0.02
7	Margaric acid	C17:0		0.25 ± 0.01
8	Stearic acid	C18:0		2.47 ± 0.08
9	Oleic acid	C18:1	n-9	51.46 ± 0.50
10	Linolenic acid	C18:3	n-3	1.52 ± 0.02
11	Linoleic acid	C18:2	n-6	30.82 ± 0.15
12	Arachidic acid	C20:0		0.19 ± 0.01
13	Eicosenoic acid	C20:1	n-9	5.75 ± 0.05
14	∑ Saturated fatty acids	-	-	10.3 ± 0.08
15	∑ Unsaturated fatty acids	-	-	89.7 ± 0.14

Values are expressed as mean ± SD of three independent GC-FID analyses (n = 3). Trace = detected below the quantification limit; – = not detected.

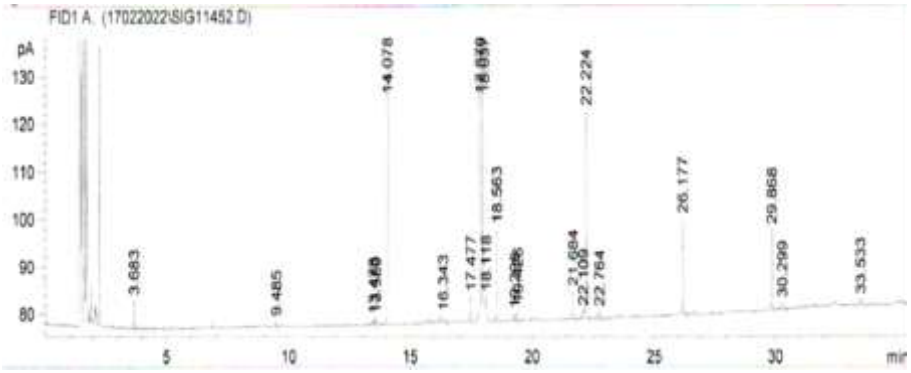


Figure 1. Gas chromatographic profile of the fatty acid composition of oil obtained from Aesculus hippocastanum L. seeds

The chromatographic profile of the identified fatty acids is presented in Figure 1, showing clear separation and dominance of oleic and linoleic acid peaks.

Antimicrobial activity

The seed oil demonstrated broad-spectrum antimicrobial effects in the agar well diffusion assay against five of seven tested conditionally pathogenic strains (**Table 2**). Strong inhibition was observed against *Staphylococcus aureus* D2 (19.87 ± 0.15), moderate against *Klebsiella oxytoca* 1 (16.80 ± 0.26), *Proteus mirabilis* 6 (15.00 ± 0.10), and *Pseudomonas aeruginosa* 003841/114 (13.93 ± 0.35), and weak against *Candida albicans* (11.50 ± 0.50). No activity was detected against *Escherichia coli* NC 101 or

Bacillus subtilis VKM. One-way ANOVA showed significant differences among the inhibition zone diameters of susceptible microorganisms ($F = 310.20$, $p < 0.001$), indicating different levels of sensitivity to A. hippocastanum seed oil.

Table 2. Antimicrobial activity of Aesculus hippocastanum L. seed oil against conditionally pathogenic microorganisms (Inhibition zone diameter (mm, mean ± SD))

No.	Strain of conditionally pathogenic microorganism	Inhibition zone diameter, (mean ± SD) mm
1	Escherichia coli NC 101	nd
2	Candida albicans	11.50 ± 0.50
3	Staphylococcus aureus D2	19.87 ± 0.15
4	Pseudomonas aeruginosa 003841/114	13.93 ± 0.35
5	Klebsiella oxytoca 1	16.80 ± 0.26
6	Bacillus subtilis VKM	nd
7	Proteus mirabilis 6	15.00 ± 0.10

Values are means of three replicates (well diameter 6 mm included, nd = no inhibition detected).

This activity pattern, which was stronger against Gram-positive bacteria (S. aureus) and variable against Gram-negative strains and yeast, mirrors reports on fatty acid-rich plant oils, where unsaturated fatty acids (particularly oleic and linoleic acids) disrupt microbial membranes by increasing membrane fluidity, inducing leakage of intracellular constituents, and inhibiting cell growth **(Štumpf et al., 2023; Dahash et al., 2021; Dridi et al., 2023).** Although S. aureus showed the highest susceptibility, B. subtilis did not exhibit detectable inhibition, indicating that susceptibility may vary among Gram-positive species. The strongest activity was observed against S. aureus (19.87 ± 0.15 mm), which may be explained by the structural characteristics of Gram-positive bacteria. Unlike Gram-negative microorganisms, Gram-positive bacteria lack an outer lipopolysaccharide membrane, allowing hydrophobic compounds present in the oil to interact more readily with the cytoplasmic membrane and exert their antimicrobial effects.

In contrast, Gram-negative strains showed moderate or no inhibition, suggesting that the outer membrane may act as an effective permeability barrier, limiting the penetration of fatty acids into the bacterial cell. This barrier function may partly explain the complete absence of activity against E. coli, despite the moderate susceptibility observed in K. oxytoca, P. mirabilis, and P. aeruginosa. Differences among Gram-negative species may also be associated with variations in membrane permeability and intrinsic resistance mechanisms, including active efflux systems and stress-response pathways. Interestingly, B. subtilis also showed no detectable inhibition. Although this species is Gram-positive, its resistance may reflect strain-specific physiological characteristics, including differences in cell wall composition, membrane structure, or adaptive responses to antimicrobial compounds **(Štumpf et al., 2023; Khar'kov et al., 2022).** While most previous studies on A. hippocastanum have focused on saponin-rich or ethanolic extracts, the present findings demonstrate that the fixed oil itself possesses measurable antimicrobial activity. This effect is likely associated with the predominance of unsaturated fatty acids, which accounted for 89.7% of the total fatty acid fraction. The observed activity therefore suggests that, in addition to well-known triterpene saponins, lipid constituents may also contribute to the biological properties of horse chestnut seeds **(Dahash et al., 2021; Dridi et al., 2023).** The overall antimicrobial activity pattern of the seed oil against the tested microorganisms is illustrated in Figure 2, confirming the greater susceptibility of S. aureus compared with Gram-negative bacteria and yeast.

Natural Resources for Human Health
eISSN: 2583-1194

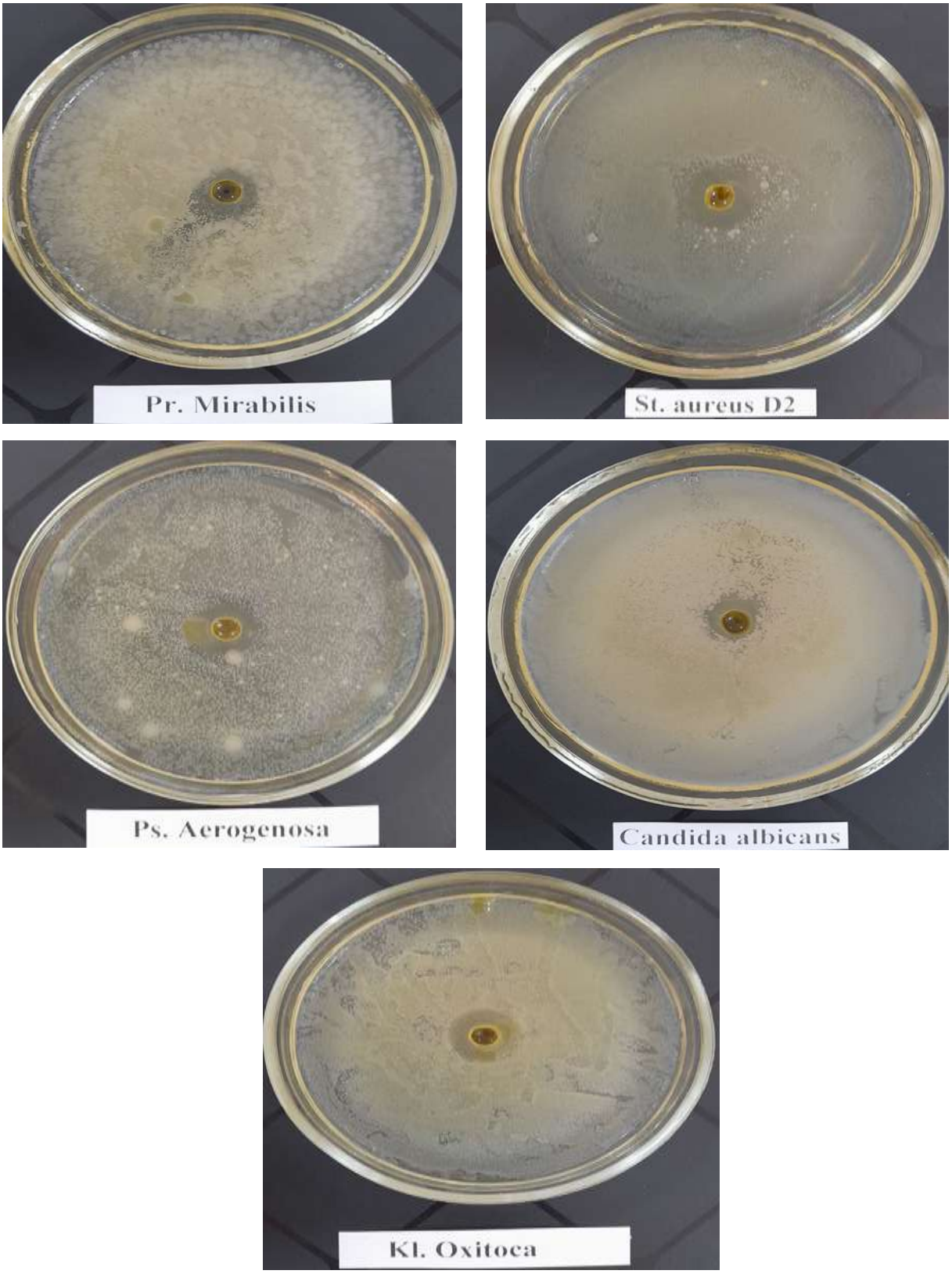


Figure 2. Antimicrobial activity of Aesculus hippocastanum L. seed oil against conditionally pathogenic microorganisms with agar gel diffusion method.

CONCLUSION

In conclusion, the seed oil from Uzbekistan-grown *Aesculus hippocastanum* represents a valuable local resource characterized by a favorable unsaturated fatty acid profile and promising in vitro antimicrobial properties, especially against Gram-positive pathogens. These findings suggest potential applications in nutraceuticals, functional foods, natural antimicrobial agents, and topical formulations. Further research, including MIC/MBC determination, mechanistic studies, stability evaluation, and formulation trials, is recommended to enable practical development and commercialization of this underutilized plant material.

AUTHOR CONTRIBUTIONS

Sh.I: Conceptualization; Methodology; Validation; Formal analysis; Investigation,

V.N: Resources; Data curation; Writing-original draft preparation,

D.R, F.N: Writing-review and editing: Visualization; Supervision,

V.A: Project administration; Funding acquisition.

A.N: Methodology; Validation; Formal analysis;

M.N: Writing-review and editing;

All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors would like to thank the staff of the Qibray district botanical garden for assistance in seed collection. This research received no external funding.

REFERENCES

[1] **Amiri, S., Shakeri, A., Sohrabi, M.R., Khalajzadeh, S., & Ghasemi, E. (2019).** Optimization of ultrasonic assisted extraction of fatty acids from *Aesculus hippocastanum* fruit by response surface methodology. *Food Chemistry*, 271, 211–217.

[2] **Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016).** Methods for *in vitro* evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6(2), 71–79.

[3] **Baraldi, C., Tait, G., Ziosi, P., et al. (2007).** Chemical composition and characterisation of seeds from two varieties of *Aesculus hippocastanum*. *Food Chemistry*, 104(1), 229–236.

[4] **Clinical and Laboratory Standards Institute. (2022).** Performance Standards for Antimicrobial Susceptibility Testing (32nd ed.). CLSI Supplement M100. CLSI.

[5] **Čukanović, J., Tešević, V., Orlović, S., et al. (2020).** Horse chestnut seed fatty acids as affected by geographic origin. *Biologica Serbica*, 42(2), 89–98.

[6] **Christie, W.W. (1993).** Preparation of ester derivatives of fatty acids for chromatographic analysis. In: *Advances in Lipid Methodology – Two*, pp. 69–111. The Oily Press, Dundee, UK.

[7] **Dahash, S. L., Al-Rawi, S. S., Al-Saffar, A. Z., et al. (2021).** β-Aescin and antimicrobial activity. *Antibiotics*, 10(4), 416.

[8] **Dridi, A., da Silva, L. P., Barros, L., et al. (2023).** *Aesculus hippocastanum* as a source of bioactive compounds. *Separations*, 10(3), 160.

[9] **Khar'kov, Y. K., et al. (2022).** Assessment of antimicrobial activity of ethanolic and aqueous extracts of *Aesculus hippocastanum* L. bark against bacteria isolated from urine of patients diagnosed positive to urinary tract infections. *Frontiers in Bioscience*, 14(2), 11.

[10] **Naghshi, S., Aune, D., Beyene, J., et al. (2021)**. Alpha-linolenic acid and mortality risk: Dose-response meta-analysis of prospective cohort studies. *BMJ*, 375, n2213.

[11] **Newman, D. J., & Cragg, G. M. (2020).** Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products*, 83(3), 770–803.

[12] **Lalpuria, M., Karwa, V., Anantheswaran, R. C., & Floros, J. D. (2013).** Modified agar diffusion bioassay for better quantification of Nisaplin. Journal of Applied Microbiology, 114(3), 663–671.

[13] **Pittler, M. H., & Ernst, E. (1998).** Horse-chestnut seed extract for chronic venous insufficiency. *Archives of Dermatology*, 134(11), 1356–1360.

[14] **Sacks, F. M., Lichtenstein, A. H., Wu, J. H. Y., et al. (2017)**. Dietary fats and cardiovascular disease: A presidential advisory from the American Heart Association. *Circulation*, 136(3), e1–e23.

[15] **Sirtori, C. R. (2001).** Aescin: Pharmacology, pharmacokinetics and therapeutic profile. *Pharmacological Research*, 44(3), 183–193.

[16] **Štumpf, S., Hostnik, G., Leitgeb, M., et al. (2023).** Antibacterial activity of chestnut extracts. *Plants*, 12(10), 2043.

[17] **Zlatanov, M. D., Antova, G. A., Angelova-Romova, M. Y., et al. (2013).** Lipid composition of horse chestnut seeds (*Aesculus hippocastanum* L.). *Journal of the Science of Food and Agriculture*, 93(5), 1196–1202.