Original Article

Antibacterial Activity and Phytochemical Analysis of *Plantago lanceolata* Root Petroleum Ether and Aqueous Extracts

Samaneh Rahamouz-Haghighi[1, 2*]

1. Agricultural Research, Education and Extension Organization (AREEO), Mashhad, Iran.
2. Department of Plant Production and Genetics, Faculty of Agriculture, University of Zanjan, Zanjan, Iran.

*** Corresponding Author:**
Samaneh Rahamouz-Haghighi, PhD.
Address: Department of Plant Production and Genetics, Faculty of Agriculture, University of Zanjan, Zanjan, Iran.
E-mail: rahamouz_haghighi.s@yahoo.com

Article info:

Received: 17 Jul 2025

Accepted: 12 Sep 2025

Keywords:

Disc diffusion method, Gas chromatography-mass spectrometry (GC-MS), Microtiter broth dilution, Minimum inhibitory concentrations (MICs), *Plantago lanceolata*, *Ribwort plantain*

ABSTRACT

Background: *Plantago lanceolata* L., a perennial plant of the Plantaginaceae family, is widely distributed across various regions. It is used globally for multiple purposes, including medicinal treatments, food additives, cosmetics, and industrial applications.

Objectives: This study aimed to investigate the antimicrobial activity and phytochemical profile of root extracts of *P. lanceolata*.

Methods: Antibacterial activity was assessed using petroleum ether and aqueous extracts of *P. lanceolata* roots. The antibacterial activity was assessed using disc diffusion and microtiter broth dilution assays to determine the minimum inhibitory concentration (MIC). Additionally, the minimum bactericidal concentrations (MBCs) were evaluated by culturing the samples on agar media. Gas chromatography-mass spectrometry (GC-MS) was also employed to identify the chemical constituents present in the extracts.

Results: The petroleum ether extract exhibited the strongest antimicrobial effect, producing an inhibition zone of 15.50 mm against *Proteus vulgaris* (PTCC 1182). The lowest MIC of 2 mg/mL was observed for both *P. vulgaris* and *Bacillus cereus* (ATCC 11778) with this extract, while minimum bactericidal concentration (MBC) values confirmed effectiveness at 3 mg/mL. Chemical analysis revealed that hexadecanoic acid ethyl ester (16.15%) and hexadecanoic acid, methyl ester (5.03%) were the predominant compounds in the petroleum ether extract. In contrast, the aqueous extract contained major artifactual components, such as 3-methoxy-2, 2-dimethyloxirane (30.78%).

Conclusion: *P. lanceolata* roots show promise as a natural antibacterial agent with potential applications across pharmaceutical, chemical, food, and medicinal industries. The antibacterial efficacy of the extracts is supported by the identification of active compounds through GC-MS analysis.

Citation Rahamouz-Haghighi S. Antibacterial Activity and Phytochemical Analysis of *Plantago lanceolata* Root Petroleum Ether and Aqueous Extracts. Pharmaceutical and Biomedical Research. 2026; 12(1):37-48. http://dx.doi.org/10.32598/PBR.12.1.1061.6

http://dx.doi.org/10.32598/PBR.12.1.1061.6

PBR
Pharmaceutical & Biomedical Research

Introduction

Plantago lanceolata L., commonly known as *Ribwort plantain*, is a widespread species within the Plantago genus of the Plantaginaceae family [1]. Although primarily a perennial, it can sometimes be classified as annual or biennial. This plant thrives in temperate regions, commonly growing in meadows, pastures, green spaces, and along roadsides [2]. Traditionally, various parts of *P. lanceolata* have been used ethnomedicinally; for example, the whole plant has been used to prepare eye lotions [3]. The aerial parts of the plant have wound-healing, anti-asthmatic, antibacterial, and anti-inflammatory properties [4]. Its leaves serve as an expectorant and are used to alleviate abdominal pain and treat inflamed wounds [5, 6]. The seeds of *P. lanceolata* were traditionally used in treating parasitic worms, and the plant's mucilage, as a laxative, reduces membrane irritation [3]. In addition to its medicinal properties, *P. lanceolata* is utilized in various industries as a food additive, in cosmetic formulations, as an ingredient in insecticides, and for environmental applications, such as the removal of heavy metals from contaminated sites. The therapeutic potential of this species is largely attributed to the rich secondary metabolite content of its leaves, roots, and bark [7]. These valuable secondary metabolites include acteoside, flavonoids, phenylpropanoid glycosides, phenylcarboxylic acids, silica, mucilage, tannins, iridoid glycosides, zinc, and potassium salts. Among the iridoid glycosides, aucubin and catalpol are particularly prominent [8]. These compounds are recognized as chemotaxonomic markers, extensively studied across multiple plant organs [9]. Previous studies have evaluated the biocompatibility and toxicity of extracts derived from different parts of *P. lanceolata* [10, 11]. Further research has focused on the biological activities of *P. lanceolata* root extracts [12]. The study of medicinal plants' chemical properties and biological potential is particularly crucial. The therapeutic effects of *P. lanceolata* are closely linked to its bioactive compounds [1]. The cost-effectiveness of petroleum ether compared to other organic solvents makes it an appealing choice as a non-polar extraction solvent [13]. It is widely utilized to extract non-polar substances such as vegetable and essential oils. *P. lanceolata* is known for its non-aromatic nature and low yield of essential oils. Previously, the aerial parts of this plant were subjected to hydrodistillation using a Clevenger apparatus, with the essential oils collected in n-pentane for further analysis [14]. Petroleum ether was identified as an effective solvent for extracting non-volatile lipophilic compounds from roots. On the other hand, aqueous extraction, using water as the solvent, is commonly applied across medicine, chemistry, and biology to isolate various compounds. This study aimed to identify the bioactive compounds and assess the antibacterial efficacy of *P. lanceolata* root extracts. Specifically, it aimed to evaluate the antibacterial properties of petroleum ether and aqueous extracts after fractionation.

Materials and Methods

Plant material

P. lanceolata was collected from the collection site (36°41'15.5"N, 48°24'02.2"E) at the University of Zanjan, Iran, and was verified by the Department of Botany, University of Zanjan. The voucher specimen number 14253 was recorded for *P. lanceolata*. The root organ of the plant was cut and shade-dried at room temperature for 7-10 days.

Extraction

A total of 250 g of powdered *P. lanceolata* roots underwent sequential extraction using petroleum ether and methanol via a reflux apparatus, with each solvent applied for 16 h. The methanol extract was further partitioned through liquid-liquid extraction with dichloromethane, ethyl acetate, n-butanol, and water [15]. The aqueous phase was filtered through Whatman No. 1 filter paper to remove fibrous plant debris (Figure 1). Only petroleum ether and aqueous extracts were selected for this study, as previously reported [12] had analyzed other fractions. The final extracts were concentrated under reduced pressure using a rotary evaporator and air-dried at ambient temperature for one week.

Microorganisms culture

Gram-positive *Bacillus cereus* (ATCC 11778) and gram-negative *Salmonella paratyphi* (ATCC 5702), and *Proteus vulgaris* (PTCC 1182) strains were obtained from the Department of Biotechnology, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran. These bacterial cultures were grown in Mueller-Hinton broth and incubated at 37 °C for 18 h before experimentation.

Antibacterial activity assay

The disc diffusion assay was used to estimate the antibacterial potential of *P. lanceolata* roots, according to the National Committee for Clinical Laboratory Standards

Table 1. GC/MS conditions to determine the volatile compounds of *P. lanceolata* extracts

GC-MS Type	Injection Volume	Column Type (mm, mm, µm)	Carrier Gas	Flow Rate	Injector and the Interface Temperature	Column Temperature Program	MS Data Libraries
Agilent Technologies GC 7890A-5975c, USA	1 µL	Capillary column (30×250×0.25)	Helium	1.0 mL/min	350 ˚C	50 ˚C (2 min)-raised to 230 ˚C at the rate of 4 ˚C/min rate (2 min)	NIST08.L

PBR

[16]. To obtain a 100 mg/mL concentration, petroleum ether and aqueous extracts were dissolved in dimethyl sulfoxide (DMSO), and the discs were impregnated with 5 µL of each extract. Gentamicin at a 10 µg/mL concentration was used as the positive control, and DMSO was used as the negative control. In this regard, the turbidity of inocula was in accordance with 1.5×10^8 CFU/mL, 0.5 McFarland standard [17]. The diameter of the inhibition zone (mm) was measured to estimate the antibacterial properties of the extracts. The Clinical and Laboratory Standards Institute (CLSI) guidelines were followed to determine the minimum inhibitory concentrations (MICs) by standard broth microdilution [18]. The root extracts were used at concentrations ranging from 1 to 4 mg/mL. MICs were determined as the lowest extract dose that did not exhibit visible growth in the well. The minimum bactericidal concentrations (MBCs) were evaluated by culturing 100 µL from each well on Mueller-Hinton agar plates and incubating at 37 ˚C for 24 h.

Gas chromatography-mass spectrometry (GC-MS) analysis

The petroleum ether and aqueous extracts of *P. lanceolata* root were analyzed by GC-MS. The conditions and temperature program are presented in Table 1, according to a previously described method [15].

Working solutions (5 mg/mL) were prepared by dissolving the dry extracts in HPLC-grade methanol. They were filtered using a sterile 0.22 µm filter before injection into the device.

Statistical analysis

All the experiments were performed in three replicates. The data were reported as Mean±SD. The Figures were designed using Excel software, version 2016.

Results

Antibacterial activity

The petroleum ether and aqueous extracts of *P. lanceolata* roots were tested individually against various bacterial strains. According to Table 2, these root extracts exhibited antibacterial activity against both gram-positive and gram-negative bacteria. The petroleum ether extract demonstrated the highest antimicrobial effect, producing a 15.50 mm inhibition zone against *P. vulgaris* using the disc diffusion method. However, none of the extracts matched the antibacterial efficacy of the standard antibiotic gentamicin against the tested bacteria (Table 2). The petroleum ether root extract had the lowest MIC of 2 mg/mL against *P. vulgaris* and *B. cereus*. These results were further supported by MBC values, which were confirmed at 3 mg/mL (Table 2). In contrast, the aqueous extract exhibited higher MICs against the tested bacteria and was less effective than the petroleum ether extract. Additionally, MBCs for the aqueous extract were not determined within the 1-4 mg/mL concentration range.

Phytochemical screening using GC-MS

Figure 2 shows the chemical groups identified in the root extracts of *P. lanceolata*. Tables 3 and 4 present detailed constituent profiles of these extracts. In the petroleum ether extract, fatty acids and esters were the dominant compounds, accounting for 57.57% of the total composition. The key components included hexadecanoic acid, ethyl ester (16.15%), palmitic acid, methyl ester (5.03%), and 9,12-Octadecadienoic acid, ethyl ester (4.72%). In contrast, the aqueous extract was characterized by a major peak of 3-methoxy-2,2-dimethyloxirane, comprising 30.78% of the extract.

Both petroleum ether and aqueous extracts contained siloxane compounds, specifically cycloheptasiloxane, tetradecamethyl-, and cyclohexasiloxane, dodecamethyl-, at varying concentrations. Notably, these compounds were more abundant in the aqueous extract (17.61% and 9.02%, respectively) compared to the petroleum ether extract (0.88% and 0.33%).

Table 2. Antimicrobial assay of *P. lanceolata* root extracts against pathogenic bacteria

Extracts (100 mg/mL)	*B. cereus* (ATCC 11778)			*P. vulgaris* (PTCC 1182)			*S. paratyphi* (ATCC 5702)		
	IZ (mm)	MIC (µg/mL)	MBC (µg/mL)	IZ (mm)	MIC (µg/mL)	MBC (µg/mL)	IZ (mm)	MIC (µg/mL)	MBC (µg/mL)
Petroleum ether	14.5±1.5^{+}	2000	3000	15.5±1.3^{++}	2000	3000	-	3000	4000
Aqueous	-	4000	R	7±0	4000	R	-	4000	R
Gentamicin (10 µg/mL)	31±2^{+++}	500	500	26±1.3^{+++}	500	500	14±1.1^{+}	500	500

PBR

Abbreviations: IZ: Diameter of inhibition zone (mm); R: Resistant (no inhibition at tested concentrations); MIC: Minimum inhibitory concentrations; MBC: Minimum bactericidal concentration.

$^{-}$No inhibition, $^{+}$Moderate activity (10–15 mm), $^{++}$Strong activity (15–20 mm), $^{+++}$Very vigorous activity (>20 mm).

Note: Extracts were prepared at 100 mg/mL in DMSO, with 5 µL (equivalent to 500 µg/disc) applied to sterile 6 mm discs for disc diffusion assay. Data represent Mean±SD of inhibition zone (mm) from three replicates. MICs and MBCs determined using concentrations from 0.5-4 mg/mL.

Discussion

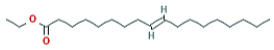
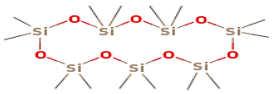
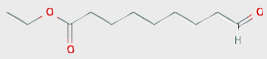
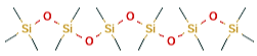
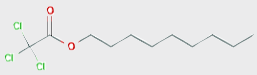
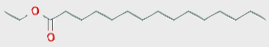
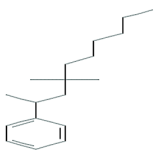
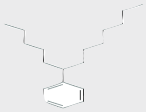
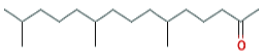
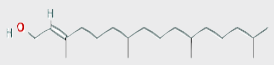
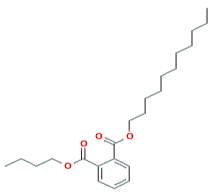
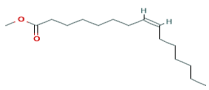
P. lanceolata is a medicinal plant valued both traditionally and in modern medicine for its ability to address a wide range of serious health conditions. Researchers have consistently confirmed its biological activities, particularly its antibacterial properties. Various studies have documented different levels of antibacterial effectiveness of *P. lanceolata* extracts against pathogenic bacteria [15, 19]. Specifically, the antimicrobial activity of the aqueous extract against *P. vulgaris* has been described as weak or moderate [20]. Similarly, in the current study, the aqueous root extract demonstrated weaker antibacterial activity than the petroleum ether extract. Another study highlighted the potent antibacterial activity of the pure petroleum ether extract from *P. lanceolata* leaves against pathogenic bacteria [21]. Consistent with these findings, our present research shows that the petroleum ether extract of *P. lanceolata* roots exhibits significant inhibitory effects on the tested bacterial strains. Our previous study showed the antibacterial effect of the dichloromethane root extract of *P. lanceolata* against *S. paratyphi* at a concentration of 100 mg/mL [12]. However, the results of this study align well with earlier research, reinforcing the antibacterial efficacy of *P. lanceolata* extracts.

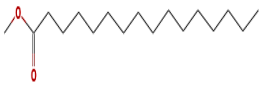
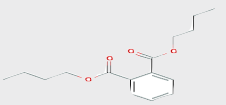
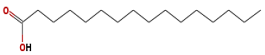
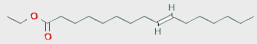
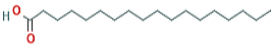
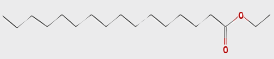
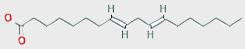
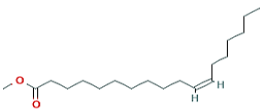
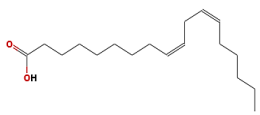
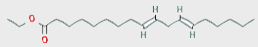
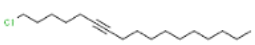
The root extracts of *P. lanceolata* exhibited distinct chemical profiles depending on the solvent used. The dichloromethane and ethyl acetate extracts were dominated by 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester, constituting 60.64% and 60.93% of their compositions, respectively. Meanwhile, the butanol extract primarily contained 2-methyl-1-butanol (±)- (17.85%) [12]. Notably, these specific compounds were absent in the petroleum ether and aqueous root extracts analyzed in this study. Comparative analysis revealed similarities between the n-hexane leaf extract of Iraqi *P. lanceolata*, which contains hydrocarbons, fatty acids, steroids, and terpenoids [22], and the Iranian petroleum ether root extract, which contains some overlapping components. Additionally, the presence of siloxane derivatives (e.g. cycloheptasiloxane and cyclohexasiloxane) in extracts is likely an artifact of gas chromatography column bleeding rather than natural plant constituents [23].

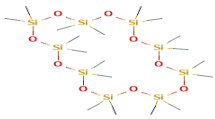
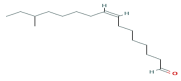
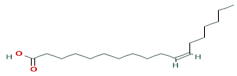
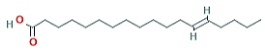
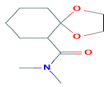
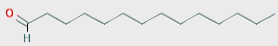
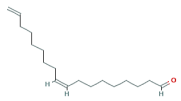
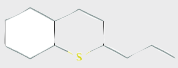
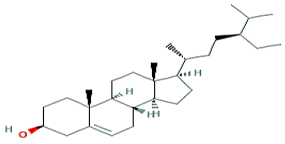
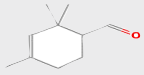
The aqueous root extract of *P. lanceolata* predominantly contained 3-methoxy-2,2-dimethyloxirane (30.78%), a low-molecular-weight epoxide also identified via GC-MS in methanolic extracts of *Cyperus alternifolius* (4.29%) [24], ethanolic extracts of *Carica papaya* L. [25], and ethanol extracts of single-use plastic bags (84.26%) [26]. The widespread occurrence of this compound across various plant and plastic-derived matrices suggests an artifactual origin, potentially arising from extraction solvents, epoxy resin degradation, or laboratory contamination. Therefore, orthogonal validation using NMR or authentic standards is crucial before assigning biological significance in phytochemical analyses [27].

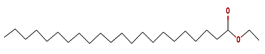
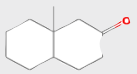
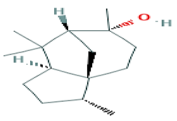
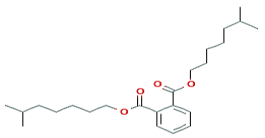
The antibacterial activity of *P. lanceolata* extracts is attributed to specific antibacterial compounds identified by GC-MS analysis. These compounds include 1,2-benzenedicarboxylic acid, diisooctyl ester [28], gamma.-sitosterol [29], hexadecanoic acid, methyl ester [30], linolenic acid [31], 9-octadecenoic acid (Z)-, methyl ester [31], Z-10-octadecen-1-ol acetate [32], octadecanoic acid [33], 9,17-octadecadienal, (Z)- [34], palmitic acid [35], pentadecanoic acid, ethyl ester [36], Phytol [37], stearic acid [38], trans-vaccenic acid [38], and other compounds.

Table 3. Compounds in petroleum ether extract of *P.lanceolata* detected by GC-MS analysis

Library/ID	RT	Area Pct (%)	Chemical Formula	Activity	Structure
(E)-9-octadecenoic acid ethyl ester or Oleic acid, ethyl ester*	3.66	3.66	$C_{20}H_{38}O_2$	Steroids and primer pheromone, perfumery, antioxidant, antiinflammatory	
Cyclohexasiloxane, dodecamethyl-	20.83	0.33	$C_{12}H_{36}O_6Si_6$	Antifungal, personal care products, emollient, lubricant, de-foaming agent	
Cycloheptasiloxane, tetradecamethyl-	25.90	0.88	$C_{14}H_{42}O_7Si_7$	Antiperspirants, deodorants, antibacterial, antifungal, antimicrobial, antiseptic, hair conditioning agent, skin- conditioning agent-emollient; solvent	
Nonanoic acid, 9-oxo-, ethyl ester	26.68	0.01	$C_{11}H_{20}O_3$	Antioxidant, antiinflammatory, anticancerous	
Hexasiloxane, tetradecamethyl-	31.08	0.16	$C_{14}H_{42}O_5Si_6$	No activity found	
Acetic acid, trichloro-, nonyl ester	32.66	0.12	$C_{11}H_{19}Cl_3O_2$	No activity found	
Octadecane*	34.50	1.82	$C_{18}H_{38}$	Antioxidant, antimicrobial, anticancer, antifungal	
Pentadecanoic acid, ethyl ester	34.40	0.74	$C_{15}H_{30}O_2$	Antibacterial, antimicrobial	
Benzene, (1,3,3-trimethylnonyl)-	34.67	0.32	$C_{18}H_{30}$	Flavoring agent	
Benzene, (1-pentyloctyl)-	35.21	0.26	$C_{19}H_{32}$	No activity found	
2-Pentadecanone, 6,10,14-trimethyl-*	35.69	0.48	$C_{18}H_{36}O$	Allelopathic, antibacterial	
3,7,11,15-Tetramethyl-2-hexadecen-1-ol or Phytol*	36.11	0.23	$C_{20}H_{40}O$	Antimicrobial, antiinflammatory	
Phthalic acid, butyl undecyl ester	36.30	0.18	$C_{23}H3_6O_4$	Antimicrobial, antibacterial, antiinflammatory	
Nonadecane*	37.01	0.33	$C_{19}H_{40}$	Antimicrobial, antibacterial, antimalarial, anti-HIVV, antioxidant, cand cytotoxic effect, for general weakness, weakness of main organs, such as Brain, heart, liver, palpitation, haemoptysis, earache, conjunctivitis, stomatitis	
9-Hexadecenoic acid, methyl ester, (Z)-*	37.40	0.29	$C_{16}H_{30}O_2$	Antioxidant	

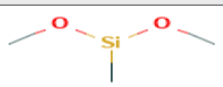
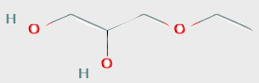
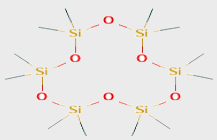
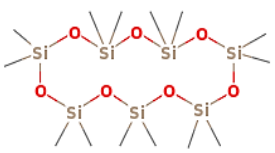
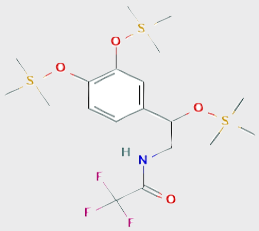
Library/ID	RT	Area Pct (%)	Chemical Formula	Activity	Structure
Hexadecanoic acid, methyl ester or palmitic acid, methyl ester*	37.56	5.03	$C_{17}H_{34}O_2$	Antibacterial, antifungal, antiinflammatory, cancer preventive, hypocholesterolemic, antieczemic, antiarthritic, hepatoprotective, insectifuge, antihistaminic, nematicide, 5-alpha reductase inhibitor, antiandrogenic, anticoronary; antioxidant, pesticide, hemolytic	
Dibutyl phthalate	38.63	1.98	$C_{16}H_{22}O_4$	Antimicrobial, as peroxisome proliferator, an endocrine disruptor with estrogenic activity; a drug channeling, Antibacterial, Antifouling	
N-hexadecanoic acid or Palmitic acid*	38.94	3.24	$C_{16}H_{32}O_2$	Antioxidant, hemolytic inhibitor, hypocholesterolemic, pesticide, nematicide, antiandrogenic, antifungal, flavour, 5-alpha reductase inhibitor, antimicrobial, antimalarial; antifibrinolytic, antialopecic	
Ethyl 9-hexadecenoate	39.06	2.07	$C_{18}H_{34}O_2$	No activity found	
Octadecanoic acid or stearic acid*	39.17	0.59	$C_{18}H_{36}O_2$	Anibacterial, antifungal, antitumor	
Hexadecanoic acid, ethyl ester or Palmitic acid ester*	39.42	16.15	$C_{18}H_{36}O_2$	Antiandrogenic, antioxidant, hypocholesterolemic, pesticide, nematicide, for flavor, lubricant, hemolytic 5-alpha reductase inhibitor	
Eicosane*	39.48	2.85	$C_{20}H_{42}$	Antitumour, antibacterial, antifungal, in the petrochemical industry	
8,11-Octadecadienoic acid, methyl ester*	41.64	2.01	$C_{19}H_{34}O_2$	No activity found	
Heneicosane*	41.74	0.75	$C_{21}H_{44}$	Antimicrobial	
9-Octadecenoic acid (Z)-, methyl ester or Oleic acid methyl ester*	41.78	2.22	$C_{19}H_{36}O_2$	Anticarcinogenic, antimicrobial, antifungal, nematicidal, and antioxidant, exist in human blood and urine; dermatitigenic flavor as endogenous peroxisome proliferator-activated receptor ligand,	
11-Octadecenoic acid, methyl ester, or trans-vaccenic acid	41.91	0.73	$C_{19}H_{36}O_2$	Antimicrobial, antioxidant	
Octadecanoic acid, methyl ester, or stearic acid methyl ester*	42.37	1.10	$C_{19}H_{38}O_2$	Antiinflammatory, anticancer, antibacterial, antifungal, antimicrobial, emulsifier, perfumery industry	
9,12-Octadecadienoic acid (Z,Z)- or linolenic acid*	42.79	1.58	$C_{18}H_{32}O_2$	Cancer preventive, antiinflammatory, antibacterial, antiarthritic, hepatoprotective, antihistaminic, anticoronary, hypocholesterolemic, antiarthritic, nematicide, antiandrogenic, antieczemic, insectifuge, antiacne, 5-alpha reductase inhibitor	
9,12-Octadecadienoic acid, ethyl ester*	43.16	4.72	$C_{20}H_{36}O_2$	Hepatoprotective, antihistaminic, hypocholesterolemic, antieczemic	
Docosane*	43.97	2.64	$C_{22}H_{46}$	Antibacterial	
3-Eicosene, (E)-*	44.30	0.16	$C_{20}H_{40}$	Antimicrobial, antihyperglycemic, cytotoxic, antioxidant, insecticidal	
6-Heptadecyne, 1-chloro-	45.62	0.17	$C_{17}H_{31}$	No activity found	

Library/ID	RT	Area Pct (%)	Chemical Formula	Activity	Structure
Cyclononasiloxane, octa-decamethyl-	45.73	0.22	$C_{18}H_{54}O_9Si_9$	Antifungal, antioxidant	
Nonadecanoic acid, ethyl ester*	45.97	4.26	$C_{21}H_{42}O_2$	No activity found	
8-Hexadecenal, 14-methyl-, (Z)-*	46.25	0.16	$C_{17}H_{32}O$	Antiinflammatory, antioxidant	
Methyl 18-methylnonadec-anoate*	46.67	1.29	$C_{21}H_{42}O_2$	No activity found	
cis-Vaccenic acid*	46.78	0.16	$C_{18}H_{34}O_2$	Antiasthmatic, antiinflammatory	
Z-10-Octadecen-1-ol acetate*	47.02	1.04	$C_{16}H_{30}O_2$	Antimicrobial	
Trans-13-Octadecenoic acid or Linoleic acid esters	47.08	0.63	$C_{18}H_{34}O_2$	Antiinflammatory, cancer preventive, antiandrogenic, dermatitigenic, ir-ritant, insectifuge, antileukotriene-D4, hypocholesterolemic, 5 anemiagenic, -alpha-reductase inhibitor, flavor	
9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)*-	47.13	0.47	$C_{20}H_{34}O_2$	Antiinflammatory, insectifuge cancer preventive, hypocholesterolemic, antihistaminic, nematicide, hepa-toprotective, insectifuge, antiacne, antieczemic, 5-alpha reductase in-hibitor, antiandrogenic, anticoronary, antiarthritic	
1,4-Dioxaspiro[4.5] decane-6-carboxylic acid, dimethylamide	47.20	0.58	$C_{11}H_{19}NO_3$	No activity found	
Tetradecanal or myristic aldehyde*	47.55	0.45	$C_{14}H_{28}O$	“Citrus-peel” flavor (when diluted), with a strong fatty, orris-like, and sweet waxy odor.	
9,17-Octadecadienal, (Z)-*	47.61	0.12	$C_{18}H_{32}O$	Antimicrobial	
Ethyl tetracosanoate*	48.13	4.68	$C_{26}H_{52}O_2$	Antimicrobial	
Tetracosane*	48.19	2.30	$C_{24}H_{50}$	Cytotoxicity, antioxidant, antibacterial, antidiarrheal, laxative, cardiotonic, anthelmintic and removes fatigue, antiinflammatory, peptic ulcer treat-ment, anticorrosive, antitrichomonas	
3-n-propyl-2-thiabicyc-lo[4.4.0]decane (cis, cis)	48.47	1.35		No activity found	
Gamma.-sitosterol*	48.77	1.43	$C_{29}H_{50}O$	Antioxidant, antibacterial, prophy-lactic	
1,3,3-Trimethylcyclohex-1-ene-4-carboxaldehyde, (+,-)-	49.35	0.12	$C_{10}H_{16}O$	No activity found	

Library/ID	RT	Area Pct (%)	Chemical Formula	Activity	Structure
Docosanoic acid, ethyl ester*	50.64	0.64	$C_{24}H_{48}O_2$	No activity found	
2(1H)-naphthalenone, octahydro-8a-methyl-, trans-	51.21	0.33	$C_{11}H_{18}O$	No activity found	
Cedrol*	51.24	0.35	$C_{15}H_{26}O$	Antimicrobial, cytotoxic	
Docosanoic acid, methyl ester*	51.69	1.15	$C_{23}H_{46}O_2$	Therapeutic, diagnostic	
1,2-Benzenedicarboxylic acid, diisooctyl ester	52.37	12.01	$C_{24}H_{38}O_4$	Antimicrobial; antioxidant	
1,21-Docosadiene*	55.28	0.74	$C_{22}H_{42}$	No activity found	

*Indicated plant-derived compounds, retention time ([RT], min), molecular weight (m/z), and relative peak area (%). PBR

Table 4. Compounds in aqueous extract of *P. lanceolata* detected by Gc-Ms analysis

Library/ID	RT	Area pct (%)	Chemical Formula	Activity	Structure
Silane, dimethoxymethyl-	3.13	18.24	$C_3H_9O_2Si$	No activity found	
3-Ethoxy-1,2-propanediol	3.37	12.82	$C_5H_{12}O_3$	No activity found	
3-methoxy-2,2-dimethyloxirane	3.61	30.78	$C_5H_{10}O_2$	Nematicide, pesticide	
Cyclohexasiloxane, dodecamethyl-	20.83	9.02	$C_{12}H_{36}O_6Si_6$	Antifungal, emollient, lubricant, de-foaming agent, personal care products	
Cycloheptasiloxane, tetradecamethyl-	26.22	17.61	$C_{14}H_{42}O_7Si_7$	Antiperspirants, deodorants, antibacterial, antimicrobial, antifungal, antiseptic, skin- conditioning agent-emollient; hair conditioning agent, solvent	
N-(Trifluoroacetyl)-O,O',O''-tris(trimethylsilyl) norepinephrine	31.08	11.50	$C_{19}H_{34}F_3NO_4Si_3$	No activity found	

Note: RT (min), Molecular weight (m/z), and relative peak area (%). PBR

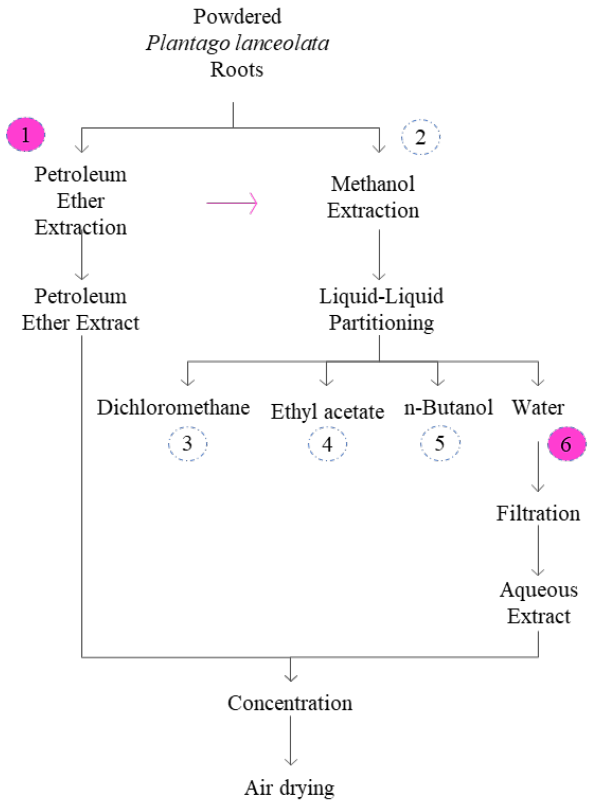


Figure 1. Flow chart of *P. lanceolata* root extraction

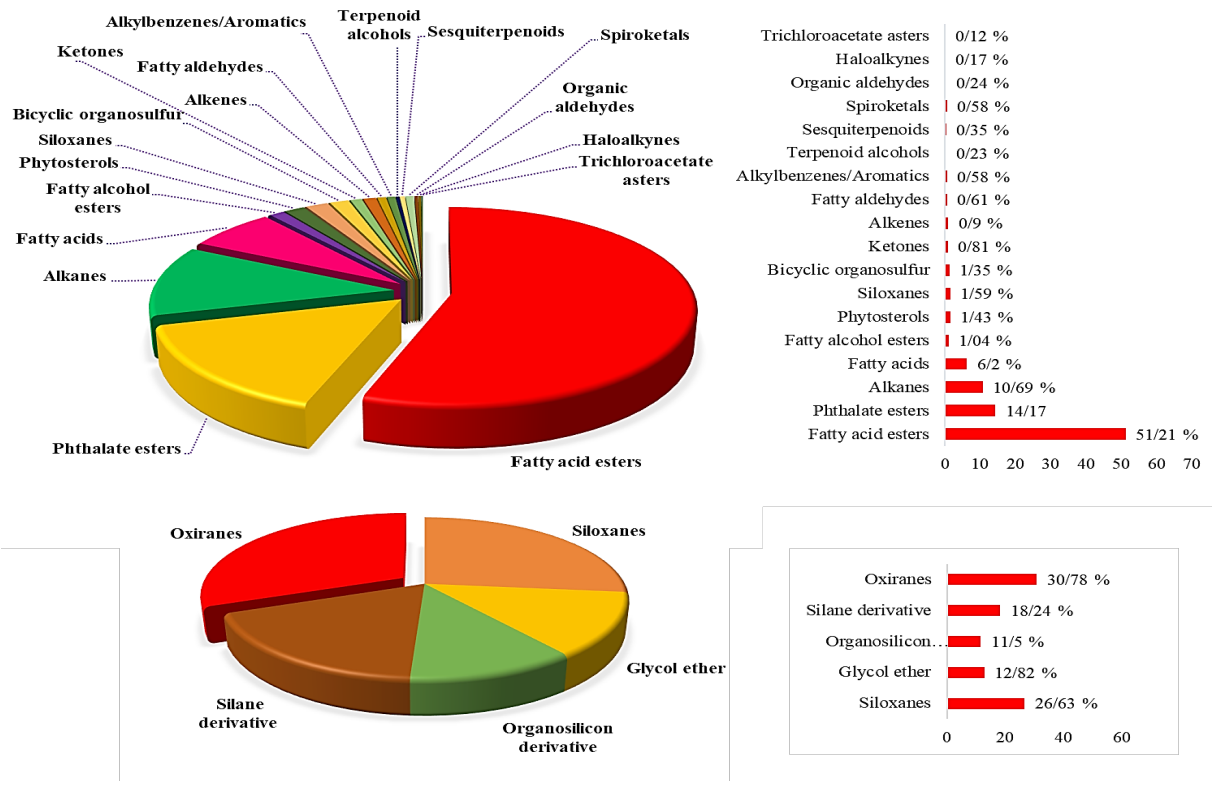


Figure 2. Different chemical groups of *P. lanceolata* root determined by GC–MS analysis
a) Petroleum ether, b) Aqueous extracts

Compounds, such as oxiranes, silanes, and siloxanes in aqueous extracts, may vary depending on the plant's geographical origin and environmental factors. However, additional studies are needed to better understand the underlying mechanisms and identify the main bioactive substances responsible for the plant's therapeutic properties.

Conclusion

Although various researchers have evaluated the antimicrobial activity of *P. lanceolata* leaves, the current study examined the antimicrobial properties of petroleum ether and aqueous extracts of *P. lanceolata* roots. Numerous compounds with antibacterial properties have been identified in *P. lanceolata*, confirming its antibacterial potential. The present research suggests conducting in vivo experiments and clinical evaluations to improve the understanding of the properties of plant roots.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

This paper was extracted from a PhD Dissertation of Samaneh Rahamouz-Haghighi, approved by the Department of Plant Production and Genetics, Faculty of Agriculture, University of Zanjan and financially supported by, University of Zanjan, Zanjan, Iran (Grant No.: A-12-848-35).

Conflict of interest

The author declared no conflict of interest.

Acknowledgments

The author appreciate the support of University of Zanjan, Zanjan, Iran.

References

[1] Abate L, Bachheti RK, Tadesse MG, Bachheti A. Ethnobotanical uses, chemical constituents, and application of Plantago lanceolata L. J Chem. 2022; 2022:1532031. [DOI:10.1155/2022/1532031]

[2] Pol M, Schmidtke K, Lewandowska S. Plantago lanceolata-An overview of its agronomically and healing valuable features. Open Agric. 2021; 6(1):479-88. [DOI:10.1515/opag-2021-0035]

[3] Pandita D, Pandita A, Pandita S. Cytogenetic exploration of P. lanceolata L. Linn.: The soldier's herb, in Jammu & Kashmir (India). Int J Bioassays. 2015; 4:4241-6. [Link]

[4] Boudjelal A, Henchiri C, Sari M, Sarri D, Hendel N, Benkhaled A, et al. Herbalists and wild medicinal plants in M'Sila (North Algeria): An ethnopharmacology survey. J Ethnopharmacol. 2013; 148(2):395-402. [DOI:10.1016/j.jep.2013.03.082] [PMID]

[5] Aziz MA, Adnan M, Khan AH, Shahat AA, Al-Said MS, Ullah R. Traditional uses of medicinal plants practiced by the indigenous communities at Mohmand Agency, FATA, Pakistan. J Ethnobiol Ethnomed. 2018; 14(1):2. [DOI:10.1186/s13002-017-0204-5] [PMID]

[6] Grigore A, Bubueanu C, Pirvu L, Ionita L, Toba G. Plantago lanceolata L. crops-source of valuable raw material for various industrial applications. Scient. Papers-Series A Agron. 2015; 58:207-14. [Link]

[7] Ahmad M, Sultana S, Fazl-i-Hadi S, Ben Hadda T, Rashid S, Zafar M, et al. An ethnobotanical study of medicinal plants in high mountainous region of Chail valley (District Swat-Pakistan). J ethnobiol Ethnomed. 2014; 10:1-18. [DOI:10.1186/1746-4269-10-36] [PMID]

[8] Mazzutti S, Riehl CA, Ibañez E, Ferreira SR. Green-based methods to obtain bioactive extracts from Plantago major and Plantago lanceolata. J Supercrit Fluids. 2017; 119:211-20. [DOI:10.1016/j.supflu.2016.09.018]

[9] Rahamouz-Haghighi S, Bagheri K, Mohsen-Pour N, Sharafi A. Quantitative determination of apigenin, catalpol, and gallic acid in total extracts from different parts of Plantago species by high-performance liquid chromatography. Avicenna J Pharm Res. 2021; 2(2):49-54. [DOI:10.34172/ajpr.2021.10]

[10] Rahamooz-Haghighi S, Bagheri K, Danafar H, Sharafi A. Anti-Proliferative properties, biocompatibility, and chemical composition of different extracts of plantago major medicinal plant. Iran Biomed J. 2021; 25(2):106-16. [DOI:10.29252/ibj.25.2.106] [PMID]

[11] Rahamouz-Haghighi S, Bagheri K, Sharafi A, Tavakolizadeh M, Mohsen-Pour N. Phytochemical screening and Cytotoxicity assessment of Plantago lanceolata L. root extracts on Colorectal cancer cell lines and Brine shrimp larvae and determination of the median lethal dose in mice. S Afr J Bot. 2022; 149:740-7. [DOI:10.1016/j.sajb.2022.06.058]

[12] Rahamouz-Haghighi S, Kh B, Mohsen-Pour N, Sharafi A. In vitro evaluation of cytotoxicity and antibacterial activities of ribwort plantain (plantago lanceolata L.) root fractions and phytochemical analysis by gas chromatography-mass spectrometry. Arch Razi Inst. 2022; 77(6):2131. [PMID]

[13] López-Olmos C, García-Valverde MT, Hidalgo J, Ferrerio-Vera C, Sánchez de Medina V. Comprehensive comparison of industrial cannabinoid extraction techniques: Evaluation of the most relevant patents and studies at pilot scale. Front Nat Prod. 2022, 1:1043147. [DOI:10.3389/fntpr.2022.1043147]

[14] Rahamouz-Haghighi S, Yazdinezhad A, Bagheri K, Sharafi A. Volatile constituents and toxicity of essential oils extracted from aerial parts of plantago lanceolata and plantago major growing in Iran. Pharm Biomed Res. 2022, 8(3):205-24. [DOI:10.18502/pbr.v8i3.11035]

[15] Jamilah J, Sharifa A, Sharifah N. GC-MS analysis of various extracts from leaf of Plantago major used as traditional medicine. World Appl Sci J. 2012, 17:67-70. [Link]

[16] NCCLS (National Committee for Clinical Laboratory Standards). Performance Standards for Antimicrobial Disk Susceptibility Test, sixth ed. Approved Standard. M2-A6, (M100-S7). Wayne: National Committee for Clinical Laboratory Standards; 1997. [Link]

[17] Rahamooz-Haghighi S, Asadi MH, Akrami H, Baghizadeh A. Anti-carcinogenic and anti-angiogenic properties of the extracts of Acorus calamus on gastric cancer cells. Avicenna J Phytomed. 2017. 7(2):145-56. [Link]

[18] Wayne P. Clinical and laboratory standards institute (CLSI). Performance standards for antimicrobial susceptibility testing. Inform Suppl. 2011; 31(1):100-21. [Link]

[19] Satish S, Mohana D, Raghavendra M, Babu S, Raveesha K. Antibacterial evaluation of some Iranian medicinal plants against some human pathogenic bacteria. Asian J Microbiol Biotech Environ Sci. 2009; 11(4):735-8. [Link]

[20] Karakaş FP, Yildirim A, Türker A. Biological screening of various medicinal plant extracts for antibacterial and antitumor activities. Turkish J Biol. 2012; 36(6):641-52. [DOI:10.3906/biy-1203-16]

[21] Abate L, Abebe A, Mekonnen A. Studies on antioxidant and antibacterial activities of crude extracts of Plantago lanceolata leaves. Chem Int. 2017; 3(3):277-87. [Link]

[22] Khalaf H A, Mahdi MF, Abaas IS. Preliminary phytochemical and GC-MS analysis of chemical constituents of Iraqi Plantago lanceoleta L. Al Mustansiriyah J Pharm Sci. 2018; 18(2):114-21. [DOI:10.32947/ajps.v18i2.485]

[23] Satpathy S, Patra A, Ahirwar B, Hussain MD. Process optimization for green synthesis of gold nanoparticles mediated by extract of Hygrophila spinosa T. Anders and their biological applications. Physica E Low Dimens Syst Nanostruct. 2020; 121:113830. [DOI:10.1016/j.physe.2019.113830]

[24] Al-Gara, Awi NI, Abu-Serag NA, Alee Shaheed KA, Bahadly ZK. Analysis of bioactive phytochemical compound of (Cyperus alternifolius L.) by using gas chromatography-mass spectrometry. IOP Sci. 2019; 571:012047. [DOI:10.1088/1757-899X/571/1/012047]

[25] Osuala O, Agunanne J, Ezemba C, Otolorin I, Enemchukwu C, Ikeh V, et al. Antimicrobial potential of aqueous and ethanol extracts of unripe Carica papaya L. Acad Nutr Diet. 2025; 2(1):1. [DOI:10.20935/AcadNutr7471]

[26] Htway EE, Htun AT, Hlaing T, Lwin MM, Min HM. Determination of chemicals released from single use low density polyethylene plastic bags. IOP Conf Ser. 2020; 496:012012. [DOI:10.1088/1755-1315/496/1/012012]

[27] Maltese F, van der Kooy F, Verpoorte R. Solvent derived artifacts in natural products chemistry. Nat Prod Commun. 2009; 4(3):1934578X0900400326. [DOI:10.1177/1934578X0900400326]

[28] Shettima AY, Karumi Y, Sodipo OA, Usman H, Tijjani MA. Gas chromatography-mass spectrometry (GC-MS) analysis of bioactive components of ethyl acetate root extract of Guiera senegalensis JF Gmel. J Appl Pharm Sci. 2013; 3(3):146-50. [DOI:10.7324/JAPS.2013.30328]

[29] Raman BV, Samuel L, Saradhi MP, Rao BN, Krishna N, Sudhakar M, et al. Antibacterial, antioxidant activity and GC-MS analysis of Eupatorium odoratum. Asian J Pharm Clin Res. 2012; 5(2):99-106. [Link]

[30] Chandrasekaran M, Senthilkumar A, Venkatesalu V. Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of Sesuvium portulacastrum L. Eur Rev Med Pharmacol Sci. 2011; 15(7):775-80. [Link]

[31] Arora S, Kumar G, Meena S. Screening and evaluation of bioactive components of Cenchrus ciliaris L. by GC-MS analysis. Int Res J Pharm. 2017; 8:69-76. [DOI:10.7897/2230-8407.08699]

[32] Subavathy P, Thilaga RD. GC-MS analysis of bioactive compounds from whole body tissue methanolic extract of Cypraea arabica (L. 1758). World J Pharm Res. 2016; 5(3):800-6. [Link]

[33] Manivannan P, Muralitharan G, Balaji NP. Prediction aided in vitro analysis of octa-decanoic acid from Cyanobacterium Lyngbya sp. as a proapoptotic factor in eliciting anti-inflammatory properties. Bioinformation. 2017; 13(9):301. [DOI:10.6026/97320630013301] [PMID]

[34] Barathikannan K, Venkatadri B, Khusro A, Al-Dhabi NA, Agastian P, Arasu MV, et al. Chemical analysis of Punica granatum fruit peel and its in vitro and in vivo biological properties. BMC Complement Altern Med. 2016; 16:1-10. [DOI:10.1186/s12906-016-1237-3] [PMID]

[35] Akpuaka A, Ekwenchi MM, Dashak DA, Dildar A. Biological activities of characterized isolates of n-hexane extract of Azadirachta indica A. Juss (Neem) leaves. Nat Sci. 2013; 11(5):141-7. [Link]

[36] Mujeeb F, Bajpai P, Pathak N. Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of Aegle marmelos. Biomed Res Int. 2014; 2014:497606. [DOI:10.1155/2014/497606] [PMID]

[37] Kumar D, Karthik M, Rajakumar R. GC-MS analysis of bioactive compounds from ethanolic leaves extract of Eichhornia crassipes (Mart) Solms. and their pharmacological activities. Pharma Innov J. 2018; 7(8):459-62. [Link]

[38] Agoramoorthy G, Chandrasekaran M, Venkatesalu V, Hsu M. Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. Braz J Microbiol. 2007; 38(4):739-42. [DOI:10.1590/S1517-83822007000400028]

This Page Intentionally Left Blank