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Original Article

in vitro Evaluation of Cytotoxicity and Antibacterial Activities of Ribwort Plantain (*Plantago Lanceolata* L.) Root Fractions and Phytochemical Analysis by Gas Chromatography-Mass Spectrometry

Rahamouz-Haghighi, S[1 *], Bagheri, Kh[1], Mohsen-Pour, N[2], Sharafi, A[2, 3]

1. Department of Plant Production and Genetics, Faculty of Agriculture, University of Zanjan, Zanjan, Iran
2. Zanjan Pharmaceutical Biotechnology Research Center, Zanjan University of Medical Sciences, Zanjan, Iran
3. Department of Pharmaceutical Biotechnology, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran

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Corresponding Author: rahamouz_haghighi.s@yahoo.com

Abstract

Ribwort plantain **(***Plantago lanceolata* L.), which belongs to the *Plantaginaceae* family, has been widely used as a herbal plant in traditional medicine across the globe. The present study aimed to investigate the biologically active substances of *P. lanceolata* root fractions, as well as the cytotoxic and antibacterial activities of extracts. The cytotoxic activity of ethyl acetate, dichloromethane, and n-butanol extracts of *P. lanceolata* root was evaluated by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. The *P.lanceolata* root extracts were also evaluated on gram-positive and negative bacteria by disc diffusion and microtiter broth dilution methods. The phytochemical content was also examined by gas chromatography-mass spectrometry. The *P.lanceolata* root extracts were cytotoxic; IC_{50} values against HCT-116 at 72 h were 168.553 µg/mL, 167.458 µg/mL, and 205.004 µg/mL for ethyl acetate, dichloromethane, and n-butanol root extracts, respectively. The dichloromethane extract of *P. lanceolata* root had the highest inhibitory effect against *S. paratyphi* (14.00±1.0 mm) at the concentration of 100 mg/mL. The minimum MIC and MBC (5 and 15 mg/mL) were observed for dichloromethane extract of *P. lanceolata* root against *S. paratyphi*. The main composition of ethyl acetate extract was 1,2-Benzenedicarboxylic acid and mono(2-ethylhexyl) ester (60.93%). The major compositions in dichloromethane and n-butanol extracts were 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester (60.64%) and 2-Methyl-1-butanol (.+/-.)- (17.85%). As evidenced by the results of the present research, *P. lanceolata* extracts are a significant source of bioactive metabolites. Therefore, they can play a prominent role in the production of pharmaceutical materials.

Keywords: Anti-cancer, HCTT-116, HEK-293, Liquid-liquid extraction, MIC, *Plantago lanceolata*

1. Introduction

Ribwort plantain (*Plantago lanceolata* L.) belongs to the Plantaginaceae family (1). This perennial medicinal herb which possesses about 275 species (2) is native to temperate regions of Asia and Europe; moreover, it can grow in moderate areas (3). Leaves, roots, and bark parts of *P. lanceolata* contain secondary metabolites with high therapeutic potentials (4). In general, *P. lanceolata* encompasses valuable secondary metabolites, such as mucilage, tannins, flavonoids, phenylpropanoid glycoside, acteoside, iridoid glycosides, phenyl carboxylic acid, silica, zinc, and potassium salts. Aucubin and catalpol are among the most common iridoid glycosides of *P. lanceolata* (5).

Numerous reports have pointed to the therapeutic features of *P. lanceolate*, including its anticancer,

antibacterial, antifungal, anthelmintic, antiviral, and antioxidant activities, in addition to the effective treatment of bee bite, colic, malaria, diarrhea, dysentery, embolism, pneumonia, tuberculosis (6, 7), and skin problems (8). Despite the growing interest in the medicinal effects of plants, there exists insufficient scientific data on the biological activities and the nature of the active compounds of herbal extracts.

P. lanceolata is a prominent source of active and beneficial metabolites, leading to its extensive use in traditional and modern medicine. Several recent studies have demonstrated that the crude extract of Plantago can exert cytotoxic effects on tumors (9-12). In light of the aforementioned issues, the present study aimed to examine the biologically active substances of *P. lanceolata* root fractions using gas chromatography-mass spectrometry (GC-MS). This study also investigated the cytotoxic and antibacterial effects of *P. lanceolata* root extract.

2. Materials and Methods

2.1. Herbal Materials

The *P. lanceolata* was collected from Zanjan, Iran (36°41'15.5"N 48°24'02.2"E) and then authenticated in the Department of Pharmacognosy, School of Pharmacy, Zanjan, Iran. The root part was cut into small pieces and dried in the shade and at room temperature for one week.

2.2. Plant Extraction

Firstly, 250 grams of *P. lanceolata* root was extracted with petroleum ether by the reflex method for 16 h. The extracts were then filtered with filter paper and washed with methanol like before. The methanolic extract was poured into the separating funnel using the liquid-liquid extraction method. The separation was performed by a funnel using ethyl acetate, dichloromethane, n-butanol, and aqueous phase (13) (Figure 1). The extracts were concentrated by a rotary evaporator and then located at room temperature to fully dry.

2.3. Cell Line Culture

Embryonic Kidney normal cell line (HEK-293) and Colorectal cancer cell line (HCT-116) were supplied from the Pasteur Institute of Iran, Tehran. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented by penicillin-streptomycin (1%) and 10% Fetal bovine serum (FBS) in an incubator (5% CO_2 and 37 °C).

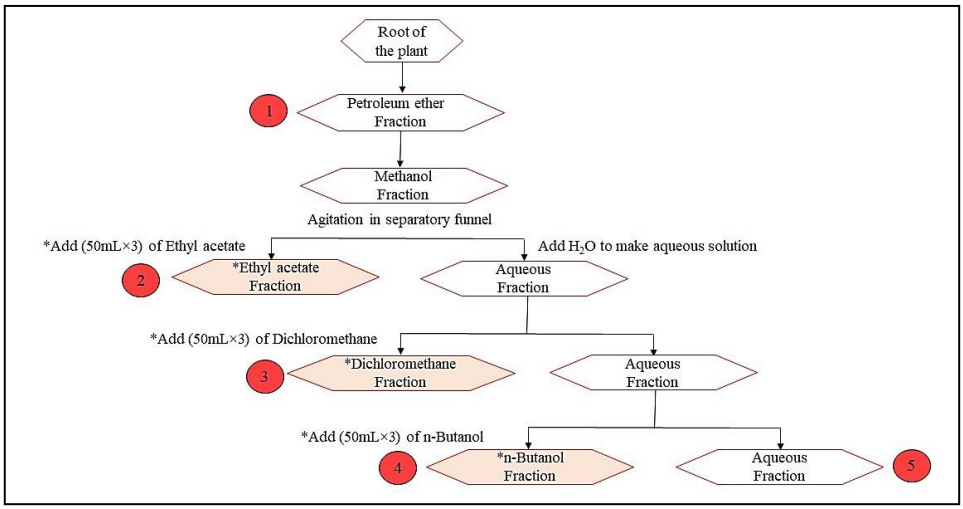


Figure 1. Flow chart of partitioning protocol of methanolic extract of *P. lanceolata root*

2.4. Viability Assay

The cytotoxicity of ethyl acetate, dichloromethane, and butanol extracts of *P. lanceolata* root was assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (14). The cells were seeded at the density of 7×10^3 cells/well. Cell attachment and growth continued overnight, followed by the dissolving of 25-400 µg/mL of the extracts. In brief, 10 mg of powdered extracts in 100 µL of Dimethyl sulfoxide (DMSO) and 900 µL of DMEM were used to make the first stock. The cells were treated by the extracts after their filtration by 0.45 µm membrane filters. Fluorouracil (5-FU) and DMSO served as positive and negative controls, respectively. After one to three days, 20 µL of MTT (5 mg/mL) was added and incubated for four h; subsequently, the medium was removed and 200 µL of DMSO was added to dissolve formazan. The absorbance of the samples was read at 570 and 690 nm using an ELISA plate reader (Tecan Infinite M200, Austria). The cell growth inhibition rates were determined by:

$$Viability(\%) = \frac{A\ sample}{A\ negative\ control} \times 100$$

where A signifies the absorbance.

2.5. Microorganism Culture

Gram-positive bacteria of *Bacillus cereus* (ATCC 10702), as well as Gram-negative bacteria of *Salmonella paratyphi* (ATCC 5702) and *Proteus vulgaris* (PTCC 1182), were prepared from the Iranian Biological Resource Centre Culture collection of bacteria.

2.6. Antibacterial Assay

The sterile blotting paper discs (6 mm diameter) were soaked in ethyl acetate, dichloromethane, and butanol extracts of *P. lanceolata* root and left to fully dry. The dried discs were used for the disc diffusion method (15). The turbidity of inoculums was matched with the 0.5 McFarland standard ($1.5\times10^*8$ CFU/mL). The bacterial inoculums were uniformly spread onto the Mueller-Hinton agar (MHA) by a sterile cotton swab. The discs were then impregnated with 3µL of extract dissolved in DMSO to obtain a concentration of 100 mg/mL. Gentamicin (10 µg/mL) and DMSO-soaked discs were considered positive and negative controls, respectively. The plates were incubated at 37°C for 24 h. Finally, the antibacterial properties were reported based on the diameter of the inhibition zone (mm).

The microtiter broth dilution method was conducted to determine the minimum inhibitory concentration (MIC) (16). 20 µL of diluted extracts at 50-250 mg/mL concentration was introduced into a 96-well plate. Thereafter, 200µL of bacterial suspensions (10^8CFU/mL) was introduced into each well and incubated at 37°C for 24 h (5-25 mg/mL desired final concentration). The absorbance of wells was recorded at 600nm using an ELISA plate reader. The MBCs were determined by culturing extracts at 15-40 mg/mL concentrations on MHA for 24 h of incubation at 37°C.

2.7. Phytochemistry

The GC-MS analysis of phytocomponents in different extracts was performed using Agilent technologies 5975c, USA. In a typical measurement, 1 µL of the ethyl acetate, dichloromethane, and butanol extracts of *P. lanceolata* root was subjected to the GC-MS system equipped with a capillary column (30 m × 250 µm ×0.25 µm, Agilent). The flow rate of Helium was 1.0 mL/min. The temperatures of the injector and the interface were set to 350°C. The following temperature program was considered: the initial column temperature was set to 50°C for 2 min, followed by an increase to 230°C at the rate of 4°C/min for 2 min. The compositions were identified by comparing the mass spectral fragmentation patterns with MS databases (NIST08.L) (13). To make the working solutions at a 5mg/mL concentration for GC/MS analysis, dried extracts were dissolved in methanol (HPLC grade), and the solutions were then filtered by a 0.22 µm sterile filter.

2.8. Statistical Analysis

The experiments were entirely carried out in triplicates. Duncan's multiple comparison test was implemented in SPSS software (version 21) ($P<0.05$)

for group-wise comparison and statistical analysis. The IC_{50} values was calculated by ED50plus software (version 1.0).

3. Results and Discussion

3.1. Cytotoxicity Activity

The colorectal cancer cell line was incubated with different concentrations of ethyl acetate, dichloromethane, and butanol extract to assess the cytotoxicity of *P. lanceolata* root. The dichloromethane extract of *P. lanceolata* root exhibited higher antiproliferative properties on HCT-116 at 24 and 48 h, as compared to ethyl acetate and butanol extracts. Nevertheless, the dichloromethane extract was more active on HEK-293 at 24 and 48 h at 200 and 400 µg/mL concentrations (Figures 2a and 2b). The proliferation inhibition activities of the cells occurred in a dose- and time-dependent manner. Three extracts of *P. lanceolata* root exhibited similar cytotoxic effects on cancer cell line at concentrations of 200 and 400 µg/mL at 72 h (Figure 2c). Moreover, 25 µg/mL of three extracts displayed no significant cytotoxicity against HCT-116 and HEK-293 (maximum 5%). These results confirmed remarkable antiproliferative properties of these three extracts of *P. lanceolata* root against HCT-116, compared to HEK-293.

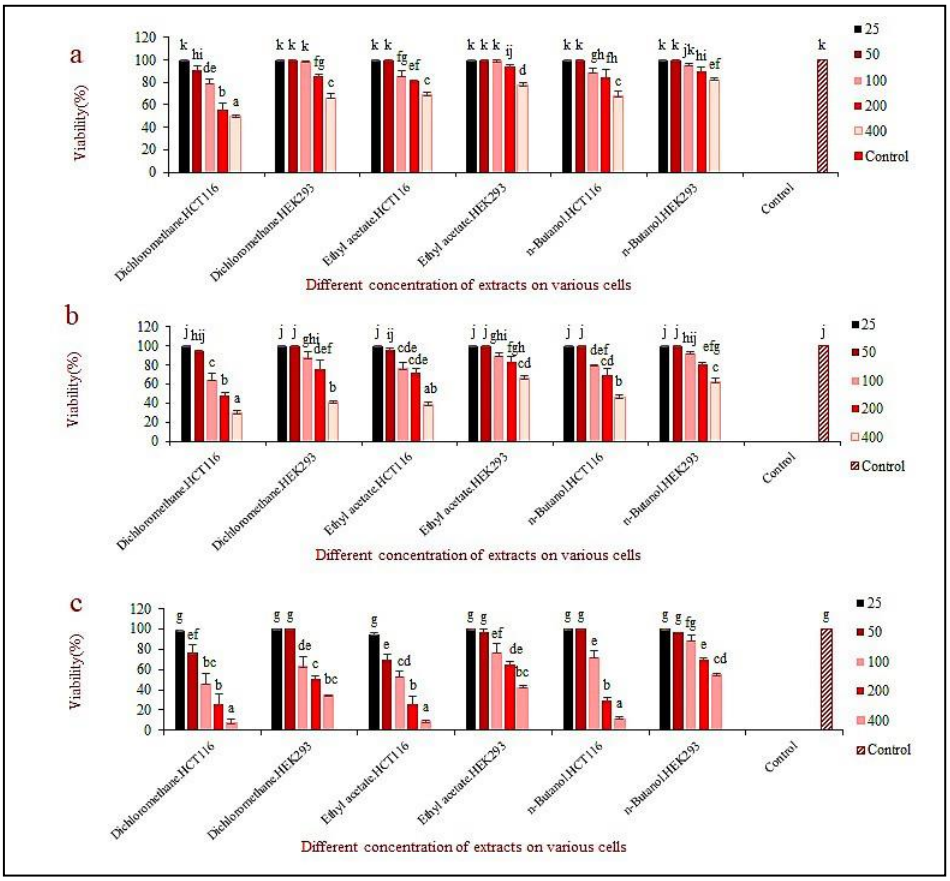


Figure 2. The MTT result for the colorectal carcinoma cell lines (HCT-116) and embryonic kidney normal cell line (HEK-293) upon treatment with ethyl acetate, dichloromethane, and butanol extracts of *P. lanceolata* root for 24, 48, and 72 h(a-c). Values represent the mean of three replications±standard deviations. Duncan test was used for mean comparison (P<0.05). Charts with the same letters are not statistically significant

As illustrated in table 1, the IC_{50} values of the ethyl acetate and dichloromethane extracts of *P. lanceolata* root (168.553 µg/mL and 167.458 µg/mL) on HCT-116 were lower than those of butanol extract (205.004 µg/mL). However, the lowest IC_{50} for *P. lanceolata* root extracts on a normal cell line was related to dichloromethane extract on HEK-293 (269.937µg/mL). Similar results have been established by *P. lanceolata* extract on breast Adenocarcinoma cell line (MCF-7) with LC_{50} of 212 µg/mL (12). In a study by Beara, Lesjak (11),*P. lanceolata* exhibited a stronger cytotoxic effect on MCF-7, cervix epithelioid carcinoma (HeLa), colon adenocarcinoma (HT-29), and human fetal lung (MRC-5) cell lines with IC_{50} values of 142.8 µg/mL, 172.3 µg/mL, 405.5 µg/mL, and 551.7 mg/mL, respectively.

Asadi-Samani, Rafieian-Kopaei (10) reported the cytotoxicity of *P. lanceolata* extracts against prostate cancer cell lines (Du-145 and PC-3) with IC_{50} values of 300 µg/mL. In this respect, Alsaraf, Mohammad (9) found IC_{50} values of 674.2 µg/mL and 23.71 µg/mL for the *P. lanceolata* extract against MCF-7 and triple-negative breast cancer cells (CAL-51), respectively. According to Rahamooz-Haghighi, Bagheri (17), IC_{50} values of methanolic, ethanolic, and acetonic extracts of *P. major* root were 470.16 µg/mL, 405.59 µg/mL, and 82.26 µg/mL, respectively, against HCT-116, while higher IC_{50} values were reported for methanolic, ethanolic, and acetonic extracts of *P. major* root toward HEK-293 (1563.04 µg/mL, 948.15 µg/mL and 125.89µg/mL, respectively). In the present research, the different extracts of *P. lanceolata* root were a proper choice for *in vitro* treatment of colon cancer cells at the concentration range of 100-400 µg/mL. In line with previous studies on *P. lanceolata*, the present research detected the cytotoxicity of extracts against cancer cells.

3.2. Antibacterial Activity

The ethyl acetate, dichloromethane, and butanol extracts of *P. lanceolata* root were evaluated on gram-positive and negative bacteria. The results indicated that the different fractions inhibited the visible growth of bacteria after 24 h (Table 2). The Dichloromethane extract of *P. lanceolata* root demonstrated the highest inhibitory activity against *S. paratyphi* (14.00±1.0 mm) at a concentration of 100 mg/mL. The dichloromethane root extract showed a similar inhibitory effect against *S. paratyphi*, as compared to gentamicin (14±1.1mm). The dichloromethane extract of *P. lanceolata* root exhibited the lowest MIC and MBC values (5 mg/mL and 15 mg/mL) against *S. paratyphi* (Table 3).

Table 1. IC_{50} of various extracts of *Plantago lanceolata* on HCT-116 and HEK-293 cell lines

Extract(µg/mL)/Cell	24h		48h		72h	
	HCT-116	HEK-293	HCT-116	HEK-293	HCT-116	HEK-293
Dichloromethane	342.23±5.8	593.46±2.8	249.06±4.2	348.76±7.8	167.45±4.6	269.93±1.8
Ethyl acetate	605.43±7.2	911.1±8.2	324.25±5.5	575.58±6.5	168.55±8.2	328.02±3.4
n-Butanol	606.04±4.3	1077.8±6.2	356.44±3.6	518.62±2.8	205.00±2.4	413.66±6.6

IC_{50} values are the mean values of three replications±standard deviations at 24, 48, and 72 h. Values were calculated for 5-fluorouracil (IC_{50}:4.136 µg/mL)

Table 2. Antimicrobial activities of *Plantago lanceolata* root extracts

Extracts (100mg/mL)	*B.cereus* (ATCC 11778)	*P.vulgaris* (PTCC 1182)	*S.paratyphi* (ATCC 5702)
Ethyl acetate	10.00±1.0	13.00±1.6	6.66±0.6
Dichloromethane	-	11.00±1.0	14.00±1.0
n-Butanol	9.00±1.0	13.50±2.0	7.00±0.5
Gentamicin (10µg/mL)	31.00±2.0	26.00±1.3	14.00±1.1

Diameter of the inhibition zone (mm), no inhibition (–)

Table 3. MICs and MBCs of different fractions of *Plantago lanceolata* root

Fractions (mg/mL)	*B.cereus* (ATCC 11778)		*P.vulgaris* (PTCC 1182)		*S.paratyphi* (ATCC 5702)	
	MIC	MBC	MIC	MBC	MIC	MBC
Ethyl acetate	20	40	10	20	10	25
Dichloromethane	25	R	20	40	5	15
n-Butanol	25	R	15	35	15	30

R: MBCs are not determined in concentrations (15-40 mg/mL)

The researchers reported that *P. lanceolata* aqueous extract illustrated poor or moderate antimicrobial activities against *S. aureus*, *S. epidermidis*, *P. vulgaris*, and *S. arcescens*; nonetheless, *P. lanceolata* methanolic extract demonstrated no significant antibacterial activity against the mentioned bacteria (18). The sensitivity of the tested bacteria to methanol, 80% and 90% aqueous-methanol, pure petroleum ether, and pure chloroform extracts of *P. lanceolata* leaves were reported by Abate, Abebe (19).

P. lanceolata extracts displayed suitable antibacterial effects against *P. vulgaris*, *B. cereus*, and *S. paratyphi*. The antimicrobial activity of *P. lanceolata* root fractions was estimated in the current study. The fractionation of methanolic extract of *P. lanceolata* root improved to separate the active compositions in *P. lanceolata* and resulted in its proper antibacterial activity. The difference in the antimicrobial properties of specific species can be attributed to the geographical region of the plant growth, extraction method, and the presence of diverse antibacterial secondary metabolites.

3.3. Phytochemical Screening

The components of *P. lanceolata* extracts were analyzed by GC-MS according to the NIST08.L library. The major aromatic constituents of *P. lanceolata* root belonged to alcohols, aldehydes, alkanes, benzofurans, fatty acids, fatty esters, phenols, phytosterols, siloxanes, and terpenoids. The highest amount of fatty acids and esters pertained to the dichloromethane fraction, which could explain the higher cytotoxic effect of this extract. Phytosterols, ethers, and phenols were found in the butanol extract. Plant-derived terpenoids are the largest class of natural products. In the current study, terpenoids were only present in ethyl acetate extract.

Following the chemical grouping of compositions in *P. lanceolata*, many biological activities have been reported for these compounds. As one of the most prominent medicinal sources, fatty acids have exhibited numerous biological activities, such as antimicrobial and antifungal activities (20), as well as anticancer behavior (21). Previous studies have stated that phytosterols could act as cytotoxic and antioxidant agents (22, 23). Phenolic compounds prevented the growth and spread of cancers *in vitro* and *in vivo* in cells and animals, respectively (24). Several studies pointed to numerous biological properties of terpenoids, including antiproliferative, antitumor, apoptotic, antimetastatic, and antiangiogenic activities (25).

The compositions of each extract of *P. lanceolata* root are presented in table 4. A number of 13 compounds were detected in ethyl acetate extract, the majority of which was 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester (60.93%). The dichloromethane extract included 24 compounds, mainly 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester (60.64%), while n-butanol extracts encompassed 18 compounds, including 1-Butanol and 2-methyl-, (.+/-.)- (17.85%). In the present study, the common components in the three extracts of *P. lanceolata* root were n-Hexadecanoic acid (3.04%, 6.073%, and 6.495%), cycloheptasiloxane, tetradecamethyl- (8.89%, 1.66% and 4.83%), and Cyclohexasiloxane, dodecamethyl- (6.58%, 0.97% and 5.89%).

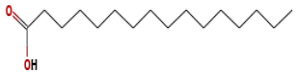
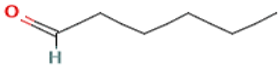
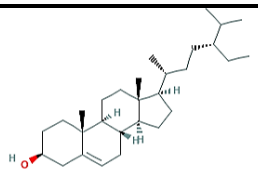
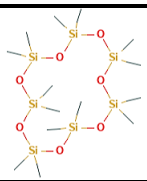
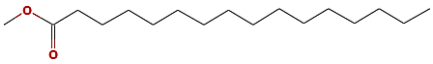
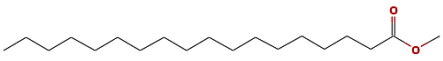
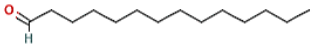
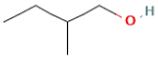
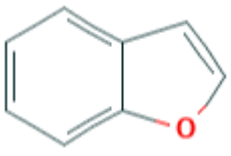
The cytotoxicity and antibacterial activities of the extracts can be ascribed to the presence of benzofuran; Cyclohexasiloxane, dodecamethyl-; Cycloheptasiloxane, tetradecamethyl-; hexadecanoic acid, methyl ester; eicosane; octadecanoic acid, methyl ester; 9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-(Linolenic acid); Cis-Vaccenic acid; hexadecanoic acid, ethyl ester, (Palmitic acid ethyl ester); Cyclotetrasiloxane, octamethyl-; trans-13-Octadecenoic acid; 1,2-Benzenedicarboxylic acid,

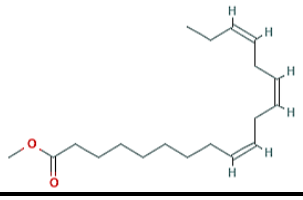
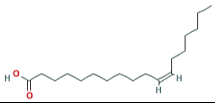
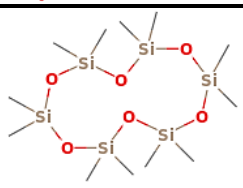
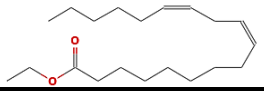
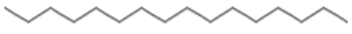
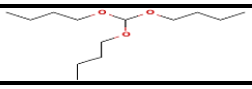
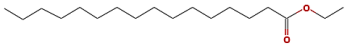
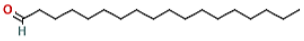
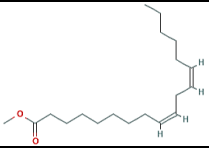
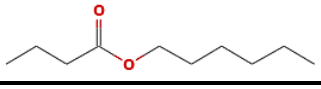
mono(2-ethylhexyl) ester; 11,13-Dimethyl-12-tetradecen-1-ol acetate, and Methyl 17-methyl-octadecanoate (Table 5). The compounds in different extracts of *P. lanceolata* root displayed numerous medicinal properties, including antibacterial, anticancer, antifungal, anti-inflammatory, antioxidant, antiparasitic, anti-yeast, and antiviral features (Table 5). The antibacterial properties of *P. lanceolata* extracts may be attributed to the presence of antibacterial compounds as reported in GC/MS analysis.

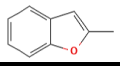
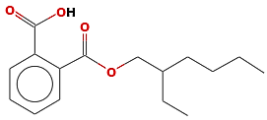
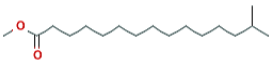
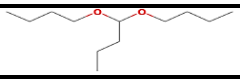
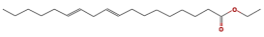
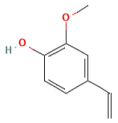
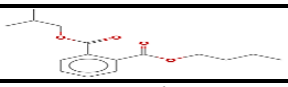
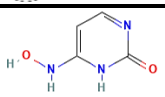
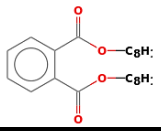
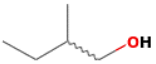
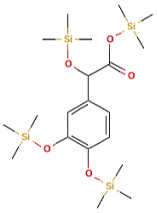
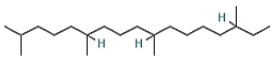
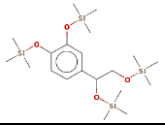
Table 4. Identification of compounds obtained by fractionation of *Plantago lanceolata* root

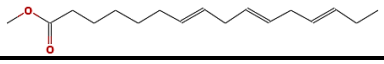
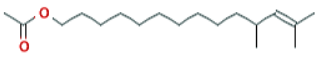
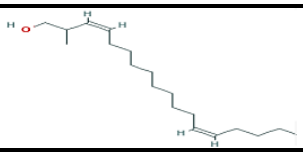
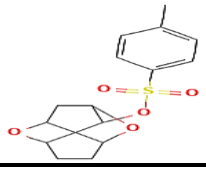
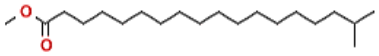
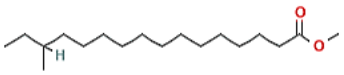
Library/ID. *Plantago lanceolata* root part	**RT (min)**	**Ethyl acetate (%)**	**RT (min)**	**Dichloromethane (%)**	**RT (min)**	**n-Butanol (%)**
n-Hexadecanoic acid	38.83	3.04	38.82	6.0734	38.74	6.4956
Hexanal	4.4	2.55	-	-	-	-
.Beta.-Sitosterol	-	-	-	-	48.15	9.07
Cycloheptasiloxane, tetradecamethyl-	26.04	8.89	26.22	1.66	26.21	4.83
Hexadecanoic acid, methyl ester	37.68	3.84	-	-	-	-
Octadecanoic acid, methyl ester	-	-	42.35	0.82	-	-
Eicosane	-	-	34.48	1.38	-	-
Tetradecanal	32.28	1.2	-	-	-	-
1-Butanol, 2-methyl-	-	-	-	-	3.59	5.52
Benzofuran	-	-	-	-	12.09	3.67
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	-	-	41.76	1.77	-	-
cis-Vaccenic acid	-	-	43.26	1.8	-	-
Cyclohexasiloxane, dodecamethyl-	20.6	6.58	20.83	0.97	20.8	5.89
Linoleic acid ethyl ester	43.12	2.25	-	-	-	-
Hexadecane	-	-	-	-	29.05	3.03
Cyclotetrasiloxane, octamethyl-	-	-	9.53	0.14	-	-
Butane, 1,1',1"-[methylidynetris(oxy)]tris-	-	-	-	-	22.95	3.67
Hexadecanoic acid, ethyl ester	39.31	3.89	39.32	4.82	-	-
Heptadecane	-	-	43.91	1.12	-	-
Nonadecane	-	-	39.4	2.03	-	-
Octadecanal	-	-	32.3	0.63	-	-
trans-13-Octadecenoic acid	-	-	43.39	0.74	-	-
9,12-Octadecadienoic acid, methyl ester	41.62	2.32	41.62	2.51	-	-
Butanoic acid, hexyl ester	-	-	-	-	22.02	1.94
Benzofuran, 2-methyl-	-	-	-	-	15.55	4.04
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	52.3	60.93	52.38	60.64	-	-
Pentadecanoic acid, 14-methyl-, methyl ester	-	-	37.68	4.39	37.67	1.9
Butane, 1,1-dibutoxy-	-	-	-	-	18.41	2.15
9,12-Octadecadienoic acid, ethyl ester	-	-	43.12	2.36	-	-
2-Methoxy-4-vinylphenol	-	-	-	-	20.65	4.36
1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	-	-	38.62	1.34	-	-
2-Hydroxy-4-hydroxylaminopyrimidine	-	-	-	-	25.68	9.54
1,2-Benzenedicarboxylic acid, diisooctyl ester	-	-	-	-	52.21	5.3
1-Butanol, 2-methyl-, (.+/-.)-	-	-	-	-	3.67	17.85
Benzeneacetic acid, .alpha.,3,4-tris[(trimethylsilyl)oxy]-, trimethylsilyl ester	-	-	31.07	0.49	-	-
Heptadecane, 2,6,10,15-tetramethyl-	-	-	48.13	0.95	-	-
Silane, [[4-[1,2-bis[(trimethylsilyl)oxy]ethyl]-1,2-phenylene]bis(oxy)]bis[trimethyl-	31.08	1.33	-	-	-	-
7,10,13-Hexadecatrienoic acid, methyl ester	41.77	1.61	-	-	-	-
O-Butylisourea	-	-	-	-	-	8.54
N-(Trifluoroacetyl)-N,O,O',O"-tetrakis(trimethylsilyl)norepinephrine	-	-	-	-	31.07	2.12
11,13-Dimethyl-12-tetradecen-1-ol acetate	-	-	35.49	0.52	-	-
2-Methyl-Z,Z-3,13-octadecadienol	43.26	1.51	-	-	-	-
Toluene-4-sulfonic acid, 2,7-dioxatricyclo[4.3.1.0(3,8)]dec-10-yl ester	-	-	48.47	0.75	-	-
Methyl 17-methyl-octadecanoate	-	-	43.83	0.86	-	-
Ethyl 14-methyl-hexadecanoate	-	-	48.07	1.15	-	-

Table 5. Compositions detected in *Plantago lanceolata* root extracts using GC/MS analysis

Library/ID. *Plantago lanceolata* root part	**Formula**	**MW (g/mol)**	**Nature of Composition**	**Biological activity**	**Structure**
n-Hexadecanoic acid (palmitic acid)	$C_{16}H_{32}O_2$	256.42	Fatty acid	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic, Antioxidant, Antifibrinolytic, Hemolytic, Antialopecic, Antimicrobial, Antifungal	
Hexanal	$C_6H_{12}O$	100.16	Fatty aldehyde	Antimicrobial, Fungicide	
Beta.-Sitosterol	$C_{29}H_{50}O$	414.7	Phytosterol	As a sterol methyltransferase inhibitor, an anticholesteremic drug, an antioxidant, a plant metabolite	
Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	519.07	Cyclic methyl siloxane	Antifungal, Skin-Conditioning Agent, Fragrance, Antimicrobial, Antifouling, Immunomodulatory, Antitumor	
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	Fatty acid methyl ester(triterpenoid)	Antioxidant, Nematicidal, Pesticidal, Hemolytic, Antiinflammatory, Cancer preventive, epatoprotective, Antihistaminic, Anticoronary, Antibacterial, Antifungal	
Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298.5	Fatty acid methyl ester	Antimicrobial, Anti-inflammatory, Anticancer	
Eicosane	$C_{20}H_{42}$	282.5	Aliphatic alkanes	Antifungal, Antitumor, Anticancer, Antibacterial	
Tetradecanal	$C_{14}H_{28}O$	212.37	Fatty aldehyde	Antibacterial	
1-Butanol, 2-methyl-	$C_5H_{12}O$	88.15	Alcohol	Antimicrobial on phytopathogen	
Benzofuran	C_8H_6O	118.13	Ether	Anti-inflammatory, Antimicrobial, Antifungal, Antihyperglycemic, Analgesic, Antiparasitic, Antitumor, Antidepressant, Anticancer, Antiviral, Antifungal, Antioxidant, Anti-psychotic	

Library/ID. *Plantago lanceolata* root part	**Formula**	**MW (g/mol)**	**Nature of Composition**	**Biological activity**	**Structure**
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-/ Linolenic acid	$C_{19}H_{32}O_2$	292.5	Fatty acid methyl ester	Anticancer, Antibacterial, Antioxidant, Antipyretic, Cardioprotective, Neural function, Antiandrogenic (5-alpha reductase inhibitor), Antiarthritic	
Cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.5	Omega-7 Fatty acid	Antibacterial, Hypolipidemic effect in rats	
Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	444.92	Cyclic methyl siloxane	Antifungal, Emollient, Personal care products, Lubricant, de-foaming agent, Antimicrobial, Antioxidant	
Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308.49	Fatty acid ethyl ester	a plant metabolite, Anti-inflammatory	
Hexadecane	$C_{16}H_{34}$	226.44	Alkane	a plant metabolite	
Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_4Si_4$	296.61	Cyclosiloxane	Antimicrobial, Antiseptic, Hair conditioning agent, Skin conditioning agent- emollient	
Butane, 1,1',1"-[methylidynetris(oxy)]tris-	$C_{13}H_{28}O_3$	232.36	Ether	Not found	
Hexadecanoic acid, ethyl ester/ Palmitic acid ethyl ester	$C_{18}H_{36}O_2$	284.47	Fatty acid ethyl ester	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic flavor, Hemolytic, Alphareductase inhibitor, Pesticide, Lubricant, 5-Alpha reductase inhibitor, antipsychotic, Antifungal, Antitumour, Antibacterial	
Heptadecane	$C_{17}H_{36}$	240.5	Alkane	Antibacterial	
Nonadecane	$C_{19}H_{40}$	268.5	Alkane	Antibacterial	
Octadecanal	$C_{18}H_{36}O$	268.5	Alpha-CH2-containing aldehyde	As the indicator of Sjogren-Larsson syndrome	
trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.46	Fatty acid	Antimicrobial	
9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.5	Fatty acid methyl ester of linoleic acid.	Hepatoprotective, Anti-histaminic, Antieczemic, Hypocholesterolemic, Antioxidant, Antimicrobial	
Butanoic acid, hexyl ester	$C_{10}H_{20}O_2$	172.26	Fatty acid ester	a potential biomarker for the consumption of these foods	

Library/ID. *Plantago lanceolata* root part	Formula	MW (g/mol)	Nature of Composition	Biological activity	Structure
Benzofuran, 2-methyl-	C_9H_8O	132.16	Ether	Not found	
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278.34	Aromatic dicarboxylic ester	Antimicrobial, Cytotoxicity, Antioxidant, Antiinflammatory, Antiviral	
Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270.5	Palmitic acid/ Fatty acid methyl ester	Antifungal, Antimicrobial	
Butane, 1,1-dibutoxy-	$C_{12}H_{26}O_2$	202.33	Ether	Not found	
9,12-Octadecadienoic acid, ethyl ester/Linolelaidic acid ethyl ester	$C_{20}H_{36}O_2$	308.49	Fatty acid ester	Hypocholesterolemic, Nematicide, Antiarthritic, Hepatoprotective, Antiandrogenic, 5-α reductase inhibitor, Antihistaminic, Anticoronary, Insectifuge, Antieczemic, Antiacne, Antimicrobial.	
2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.17	Phenol	A pheromone, a flavoring agent, a plant metabolite	
1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	$C_{16}H_{22}O_4$	278.34	Ester	Antiviral, Antimicrobial	
2-Hydroxy-4-hydroxylaminopyrimidine	$C_4H_5N_3O_2$	127.10	Not determined	Not found	
1,2-Benzenedicarboxylic acid, diisooctyl ester/ phthalate ester	$C_{24}H_{38}O_4$	390.6	Fatty acid ester, diester	Antioxidant, Antimicrobial	
1-Butanol, 2-methyl-, (.+/-.)-	$C_5H_{12}O$	88.14	Alcohol	Antimicrobial, Antiyeast	
Benzeneacetic acid, .alpha.,3,4-tris[(trimethylsilyl)oxy]-, trimethylsilyl ester	$C_{20}H_{40}O_5Si_4$	472.9	Ester	Chronic oral toxicity study of erythritol in dogs	
Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	296.6	Alkane	Antituberculous activity along with other pharmacological activities	
Silane, [[4-[1,2-bis[(trimethylsilyl)oxy]ethyl]-1,2-phenylene]bis(oxy)]bis[trimethyl-	$C_{20}H_{42}O_4Si_4$	458.9	Alkane	Not found	

Library/ID. *Plantago lanceolata* root part	**Formula**	**MW (g/mol)**	**Nature of Composition**	**Biological activity**	**Structure**
7,10,13-Hexadecatrienoic acid, methyl ester	$C_{17}H_{28}O_2$	264.40	Fatty acid methyl ester	Not found	
O-Butylisourea	-	-	Not determined	Not found	Not found
N-(Trifluoroacetyl)-N,O,O',O"-tetrakis(trimethylsilyl)norepinephrine	$C_{22}H_{42}F_3NO_4Si_4$	553.9	Not determined	Not found	Not found
11,13-Dimethyl-12-tetradecen-1-ol acetate	$C_{18}H_{34}O_2$	282.5	Alcohol	Antioxidant, Antitumour	
2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280.5	Terpenoid	Pesticide, Herbicide, Insecticide, Pheromone	
Toluene-4-sulfonic acid, 2,7-dioxatricyclo[4.3.1.0(3,8)]dec-10-yl ester	$C_{15}H_{18}O_5S$	310.4	Not determined	Not found	
Methyl 17-methyl-octadecanoate	$C_{20}H_{40}O_2$	312.5	Ester	Antimicrobial, Antioxidant, Antitumor	
Ethyl 14-methyl-hexadecanoate	$C_{18}H_{36}O_2$	284.5	Ester	Not found	

The study by Jamaluddin, Sharifa (13) exhibited various main constituents in *P. major* leaf extracts, including ethyl acetate extract (30.70% glycerin; 21.81% benzene and 16.22% dibuthyl phthalate) and n-butanol extract (24.62% phthalic acid; 16.83% benzene propanoic acid and 10.20% phenol group). In our previous study, GC-MS analysis detected octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13, 15,15-hexadecamethyl- (4.97%); cyclohexasiloxane, dodecamethyl- (6.35%); verbenone (6.96%); isoborneol (8.68%); tetradecamethylcycloheptasiloxane (9.74%); and n-hexadecanoic acid (13.8%) in the methanolic extract of *P. major* root (17).The results of the current study on the Iranian *P. lanceolata* demonstrated similarities and differences in the amounts and types of compounds observable in the extracts.

4. Conclusion

As evidenced by the results of the present research, *P. lanceolata* extracts are a significant source of bioactive metabolites. Therefore, they can play a prominent role in the production of pharmaceutical materials and the development of anticancer drugs. In general, the results of the current study highlight the potential use of various fractions of *P. lanceolata* as a source of cytotoxic agents. *P. lanceolata* extracts possess antibacterial properties and could be employed as a natural antibacterial agent to control pathogenic strains. These results are particularly important in the case of human pathogenic infections, such as *P. vulgaris*, *S. typhi*, and *B. cereus*.

Authors' Contribution

Project administration, Investigation, Formal analysis, and Writing – original draft: S. R. H.
Funding, Supervision: Kh. B.
Funding, Supervision, Conceptualization: A. S. H.
English edit: N. M. P.

Ethics

The above-mentioned sampling/treatment protocols obtained approval from the University of Zanjan

Research Ethics Committee, and Zanjan University of Medical Sciences, Zanjan, Iran (ethical code: A-12-848-35).

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgment

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