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BIOCOMPATIBILITY, CYTOTOXICITY AND PHYTOCHEMICAL ANALYSIS OF *PLANTAGO LANCEOLATA* L. AERIAL EXTRACTS: *IN VITRO* AND *IN VIVO* STUDY

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ABSTRACT

Plantago lanceolata L. is classified as Plantaginaceae family. Its bioactive properties have been documented in scientific literature, suggesting its efficacy in therapeutic interventions across a spectrum of medical conditions, with a particular focus on cancer treatment. The acetonic and alcoholic extracts of *P. lanceolata* aerial organs were subjected to the MTT technique, *Artemia salina*, oral acute hemolysis, GC-MS, and phytochemical screening to determine their *in vitro* cytotoxic impact, *in vivo* toxicity, biocompatibility, and phytochemical screening, respectively. The *P. lanceolata* acetonic extracts exhibited the lowest IC_{50} values on HCT-116 and HEK-293 cells. Between 185.04 and 123.98 µg/mL. *P. lanceolata* extracts in methanol and ethanol showed no toxicity against *A. salina* (LC_{50}: 27.25 mg/mL and 14.42 mg/mL). For four hours, the tested dosages of the alcoholic extract on red blood cells showed no signs of toxicity. One week following treatment, ethanolic and methanolic extracts of *P. lanceolata* were not deadly when taken orally. On the Hodge and Sterner scale, *P. lanceolata* extracts showed no indications of toxicity. *P. lanceolata*'s methanolic extract was described by its primary chemical elements, including n-hexadecanoic acid (15.00%); octadecanoic acid (9.80%); cis-vaccenic acid (5.66%), and 2,3-dihydroxysuccinic acid (5.66%). *P. lanceolata* acetonic extract contained n-hexadecanoic acid(1.53%), oleic Acid (1.34%) and linoleic acid (1.15%). The methanolic and ethanolic extracts did not induce hemolysis and were more cytotoxic against HCT-116 compared to HEK-293. From a pharmaceutical point of view, if toxic drugs show selective toxicity against cancer cells and are non-toxic against normal cells, it is considered advantageous.

KEYWORDS: Acute Oral Toxicity, Brine Shrimp Larvae, Colorectal Cancer Cell, Hemolysis, Human Embryonic Kidney Cell, GC-MS.

INTRODUCTION

CRC (Colorectal cancer) is the third most fatal disease on a global scale, affecting an estimated population of 1.2 million people (1). CRC is much less common When comparing Iran's senior population to that of Western nations, including the United States and European countries, However, the frequency of this occurrence is on the rise among the younger generation in Iran (2). According to the GLOBOCAN database, (2018), the CRC incidence in Iran will double until 2040 (3).

The researchers are interested in the study of medicinal plants because they confirmed many applications in pharmacy and medicine (4). There are 400 species in the Plantago genus, which is a member of the Plantaginaceae family. Meanwhile, some species are found as cosmopolitan weeds (5). However, they are known for a diverse range of applications in pharmaceutical and edible purposes, such as in salads, cooking, and animal feed (6). Chemical components obtained from the leaf, stem, and root of the Plantago genus have demonstrated therapeutic effects (7).

P. lanceolata L., commonly referred to as narrow-leaved plantain, is a widely recognized perennial species of the Plantago genus. Although it is occasionally classified as annual or biennial, it is generally characterized by a large number of shallow fibrous roots and a smaller number of deep roots. Additionally, this species is typically pollinated by the wind. Its prevalence is notable in green areas, meadows, pastures, and even roadside strips

throughout temperate regions. (8, 9). P. lanceolata has been reported to treat various diseases such as blood circulation, cancer, digestive organs, inflammation, wound healing, reproductive system, and respiratory disorders (8). As well, this plant can also be applicated for insecticide (10) and as metal removal from polluted areas (11). P. lanceolata extracts exhibited various properties such as antioxidant (12), anti-inflammatory (13), antibacterial (14), viscoelastic and rheological (15). There are different chemical constituents in the leaf, flower, root, and seed of P. lanceolata such as polyphenols, flavonoids, polysaccharides, iridoid glycosides (7), tannins (16), cinnamic acids and lipids (17), coumarins (18). These constituents displayed therapeutic potential (7).

In Iran, many seed plants have medicinal properties. These plants have the potential to be cultivated as vegetables, but the biological activities of their aerial and underground organs have not been sufficiently studied. Therefore, researchers are interested in the assay biological activity of their extracts. Plantago species are distributed as landraces accessions in different regions of Iran. To improve our understanding of the medicinal potential of Plantago sp., it is important to conduct comprehensive studies on the biocompatibility, cytotoxicity assay, and phytochemistry of P. lanceolata aerial organs extracts.

MATERIAL AND METHODS

HERBAL MATERIALS

The University of Zanjan in Iran is where the aerial organs of the wild P. lanceolata were obtained. (36°41'15.5"N 48°24'02.2"E). The Department of Pharmacognosy at the School of Pharmacy in Zanjan, Iran identified the collection of plants and assigned it a voucher specimen number of 14253. The plant materials were air-dried in a shaded area and afterward, were separately pulverized into a fine powder. Twenty g of powder of P. lanceolata aerial organs were weighed for extraction by reflux using the addition of 200 mL of acetone for 3 h followed by ethanol or methanol for 8 h. After filtration, the extracts from the plant were concentrated using a rotating vacuum-based evaporator. After that, the extracts were left to completely dry for a week at room temp in a shaded area. (19,20).

IN VITRO CYTOTOXICITY ASSAY

The Pasteur Institute of Iran in Tehran provided two cell lines for the study: HCT-116 Human Colorectal Cancer cells and HEK-293 Kidney of a human embryo cells were utilized as a standard cell line, while HCT-116 cells were utilized to represent a cancer cell line. The cells were cultivated using Dulbecco's Modified Eagle Medium (DMEM). A fetal bovine serum concentration of 10% and 1% penicillin-streptomycin were added as supplements. This culture was incubated at 37 °C in an atmosphere that was humidified with 5% CO2. To assess the potential Toxicity of acetonic and methanolic extracts of P. lanceolata, Assays such as MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were used to evaluate the cytotoxicity (21). 96-well plates were seeded with 7 × 103 cells per well. The cells were allowed to connect overnight. To prepare a 10mg/mL stock, 10 milligrams of extracts were dissolved in about 900 µL of DMEM and 100 µL of DMSO. Next a 1mg/mL stock we prepared by diluted a 10mg/mL stock in DMEM. The considered concentrations (25 to 400 µg/mL) were created using the dilutions of 1mg/mL stock. Subsequently, the cells were subjected to treatment with the extracts, while DMSO was used as a negative control at identical concentrations employed for diluting the extracts. The cells were incubated for one-three days. 5 mg/mL MTT (20µL) was introduced into the wells and allowed to incubate for 4 h. DMSO was used to replace the content of each well. The absorbance was measured at 570 nm in wavelength while the background noise was assessed at 690 nm. The cell viability rate was examined using this formula:

(A) indicates absorbance abbreviation.

BIOCOMPATIBILITY ASSAY (HEMOLYSIS TOXICITY)

In the current study, the hemolysis assay was performed for the investigation of the biocompatibility of alcoholic extracts of P. lanceolata (19). Ethylenediaminetetraacetic acid-containing tubes were used to capture freshly provided human red blood cells (RBCs). Following a 5-minute centrifugation at 1663 × g, the samples were cleaned using an isotonic saline solution (pH 7.4, PBS). Re-suspending erythrocytes tube in the same medium resulted in a 5% hematocrit, and then alcoholic extracts were added to diluted human RBC suspension (0.4 mL) at 25 to 400 µg/mL concentrations. After shaking the samples in suspension, they were all incubated for four hours at 37°C. For five minutes, the samples were centrifuged at 5400 × g in order to extract the non-lysed human red blood cells. The supernatant of the sample tube (100 µL) was transferred into a 96-well plate. The supernatant was then utilized to assess hemoglobin release at a wavelength of 545 nm. PBS and sodium dodecyl sulfate (0.1%) were the negative and positive controls to induce 0% and 100% hemolysis. Hemolysis (%) was measured using this formula:

ASSESSMENT OF CYTOTOXIC ACTIVITY OF P. LANCEOLATA USING A. SALINA LETHALITY BIOASSAY

Cytotoxicity of ethanolic and methanolic extracts was examined on brine shrimp larvae (A. Salina Leach) (22). A. salina's eggs were provided from Urmia University in West Azerbaijan, Iran.The cysts were cultivated in a flask that contained distilled water with 35 g/L of sodium chloride and incubated for 48 hours at 28 °C. The incubation condition was forcefully aerated and with continuous illumination to hatch the nauplii within 48 h. Next, 200 microliters of RPMI-1640 and 20 µL of methanolic and ethanolic extracts (7.8125 µg/mL to 1000 µg/mL) were pipetted into each well after 10 nauplii were collected. Incubation of the plates lasted for 24 hours at 25 °C. One day later, a binocular microscope was used to examine the live nauplii in each well. In each well, ten nauplii and synthetic saltwater served as the negative control. The percentages of nauplii mortality in the test and control wells were used to determine the fatal concentration. Abbott's formula was used to calculate lethality:

IN VIVO CYTOTOXICITY ASSAY

Ten Swiss Albino mice weighing between 25 and 35 g were given by Zanjan University of Medical Sciences. The mice were of both sexes. For a week, the mice were kept in cages made of filtered polycarbonate and allowed to get acquainted to the circumstances of the lab. They received standard food and drink throughout this time, in accordance with the OECD's requirements. An acute oral toxicity test was conducted in order to determine the LD50 value and assess the P. lanceolata methanolic and ethanolic extracts' safety. (23). The mice (n = 5) were separated into groups at random and given oral dosages of P. lanceolata extracts. Utilizing a feeding tube, the animals were given oral gavage with P. lanceolata extracts at methanolic and ethanolic doses ranging from 250 to 2000 mg/kg. The mice were monitored for physical activity levels and any general behavioral alterations. After ensuring the survival of all mice for 24 hours, two more mice were subjected to the maximum dose of 2000 mg/kg for treatment. It was concluded that the LD50 exceeded the chosen dose since both mice survived, and the experiment was terminated. At 24 and 168 hours, the weights of the treated animals as well as the control group were recorded. All of the mice were put to death at the conclusion of the trial.

PHYTOCHEMICAL ANALYSIS

The methanolic and acetonic extracts of P. lanceolata was examined using the Agilent Technologies 5975c gas chromatography-mass spectrometry (GC-MS) from United States. The capillary column of Agilent (30 m × 250 µm ×0.25 µm) of the GC-MS system was filled with 1 µL of extracts diluted at a rate of 5 mg/mL. At 1.0 ml/min, the flow rate of helium was maintained. Both the injector and interface temperatures were maintained at 350°C. The following circumstances applied to the temperature settings: beginning with a column temperature of 50 °C for two minutes, and rising for two minutes to 230 °C at a rate of 4 °C/min. The constituents were identified by cross-referencing mass spectrum fragmentation patterns with the NIST08.L mass spectrometry library. (24)

STATISTICAL ANALYSIS

The data is displayed as the average of three repetitions ± standard deviation. One-way ANOVA and Duncan's multiple range test were used in SPSS version 21 to do a group-wise comparison with a p-value of less than 0.05. The IC50 and LC50 values were estimated using ED50 plus v1.0 software.

RESULTS

CYTOTOXICITY ACTIVITY OF P. LANCEOLATA EXTRACTS ON CELL LINES

The methanolic and acetonic extracts of P. lanceolata aerial organs were tested for cytotoxicity using human colorectal cancer cells. It was clear that the cells' effects were time- and dose-dependent. At 200 µg/mL for 24 and 72 hours, the methanolic extract from P. lanceolata aerial organs showed more cytotoxicity against cancer cells than normal cells ($p < 0.05$, Figure 1a-c). After 24-48 hours, 400 µg/mL of P. lanceolata's ethanolic extract had the most cytotoxic effects on cancer cells ($p < 0.05$, Figure 1d-f). The alcoholic extracts exhibited lower cytotoxicity on cancerous and non-cancerous cells than acetonic extract. The cytotoxic activity of both alcoholic extracts of P. lanceolata aerial organs on normal cells at a concentration of 400µg/mL was calculated between 1-44% after 24 to 72h. The acetonic extract showed more cytotoxic effects on HEK-293 and HCT-116 for 24 to 72 h compared to alcoholic extracts ($p < 0.05$, Figure 1g-i). Nevertheless, P. lanceolata acetonic extract displayed the highest antiproliferative effects against HEK-293 at 72 h. Indeed, The acetonic extracts of P. lanceolata showed the lowest IC50 against HCTT-116 and HEK-293 cells (185.04 and 123.98 µg/mL) (Table 1). IC50s of both alcoholic extracts were almost similar.

HEMOLYSIS TOXICITY

Erythrocyte hemolysis was an index to predict the cytotoxicity of extracts. The alcoholic extracts exhibited no toxicity at the doses tested for 4 hours (Figure 2).

CYTOTOXIC ACTIVITY OF P. LANCEOLATA AGAINST A. SALINA

Both methanolic and ethanolic extracts of P. lanceolata were not determined as toxic agents against A. salina (LC50 > 1000 µg/mL). At 7.8125-500µg/mL concentrations of alcoholic extracts, all of the larves were alive, and at the highest dose, the percentage of lethality was between 1 and 2 % (LC50:27.25mg/mL for methanolic extract and 14.42 mg/mL for ethanolic extract) (Figure 3).

ORAL ACUTE TOXICITY ASSAY

The body weight serves as an effective indicator for studying the toxic effects of extracts. After 24 and 196 hours, the animal's weights fluctuated normally. Oral administration of methanolic and ethanolic P. lanceolata extracts did not result in fatality one week after therapy.

The Hodge and Sterner toxicity scale has been used to establish that P. lanceolata preparations are non-toxic (Table 2).

PHYTOCHEMICAL SCREENING

GC-MS analysis was aimed at determining the phytochemical constituents. The chemical compositions of aerial alcoholic extracts of P. lanceolata were appraised according to the NIST08.L library. n-hexadecanoic acid (15.00%); octadecanoic acid (9.80%); cis-vaccenic acid (5.66%) and 2,3-dihydroxysuccinic acid (5.66%) were the components that stood out the most in P. lanceolata's methanolic extract. acids octadecanoic and n-hexadecanoic and cis-vaccenic acid are fatty acids, and 2,3-dihydroxysuccinic acid is a dihydroxy derivative of succinic acid, its properties are listed in Table 3. Palmitic acid (1.53%) fatty acid, oleic Acid (1.34%) ester and linoleic acid (1.15%) were also the main components in P. lanceolata acetonic extract (Table 4). The present study determined that n-hexadecanoic acid, 9,12-octadecadienoic acid (Z,Z)-, tetradecamethylcycloheptasiloxane, hexadecanoic acid, methyl ester, and cyclohexasiloxane, dodecamethyl, were among the common ingredients found in the acetonic and methanolic extracts of P. lanceolata aerial organs. However, siloxanes may result from contamination in the GC column rather than in the extract itself. The origin of phthalates in P. lanceolata is unclear. It's important to note that siloxanes, phthalates, and halogenated compounds are unlikely to be natural products

The components in various extracts of P. lanceolata aerial organs have been found to possess a wide range of medicinal properties, such as antimalarial, antifungal, antioxidant, antitumor, anticancer, antimicrobial, hepatoprotective, antiplatelet, anti-inflammatory, anti-coronary, and more. These properties have been listed in Tables 3 and 4.

Cell/extracts	24h			48h			72h		
HCT-116	Methanolic	Ethanolic	Acetonic	Methanolic	Ethanolic	Acetonic	Methanolic	Ethanolic	Acetonic
	1502.97	7255.14	422.04	942.24	935.81	243.17	309.62	343.90	185.04
HEK-293	Methanolic	Ethanolic	Acetonic	Methanolic	Ethanolic	Acetonic	Methanolic	Ethanolic	Acetonic
	2195.79	51739.10	239.28	869.96	2321.43	151.72	474.08	455.81	123.98
Selectivity index	1.46	7.13	0.56	0.92	2.48	0.62	1.53	1.32	0.67

IC_{50} values are the mean of three replications ± standard deviation at 24, 48, and 72 h.

Table 1. IC_{50} values of various extracts of Plantago lanceolata aerial organs against HCT-116, and HEK-293 cell lines

Extracts	Dose (mg/kg)	Mean weight of animals (24hrs)	Duration of study (one week)	Dead rate (%)
Methanolic	17.5	30±1.27	32±0.79	0
	175	35±1.10	36±1.27	0
	1750	38±1.27	37±1.58	0
	2000	29±1.58	31±1.30	0
Ethanolic	17.5	30±1.21	31±1.27	0
	175	35±1.64	34±1.15	0
	1750	34±2.23	35±1.45	0
	2000	26±0.70	28±1.33	0

Table 2. Weight Change and Mortality Rates of Animals Submitted to Various Doses of Methanolic and Ethanolic Extract of Plantago Lanceolata Aerial Organs.

RT	Area Pct	Library/ID (aerial organ of. *P. lanceolata)*/ Synonym	Formula	M.w (g/mol)	Biological activity
9.1665	0.1788	1,2-Diethyldiborane-D10	nd	nd	Not found activity
33.262	0.1106	3-(Methylthio)-1-propanamide	nd	nd	Not found activity
38.81	15.0089	*n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Hemolytic inhibitor, Antiandrogenic
43.763	9.8006	*Octadecanoic acid	$C_{18}H_{36}O_2$	284.5	Inflammatory, Anticancer, Antibacterial
24.684	0.1021	2(1H)-Quinolinone	C_9H_7NO	145.16	Not activity found
42.594	0.9558	*9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4455	Antimicribial, Antifungal, Hepatoprotective, Antihistaminic, Antiinflammatory, Cancer preventive, Antiandrogenic, Nematicide, Antioxidant
14.98	0.1838	Formamide, N,N-dimethyl-	C_3H_7NO	73.0938	Not activity found
25.226	0.1593	Ethanamine/ Aminoethane	C_2H_7N	45.085	Not activity found
44.624	3.5014	Phenol, 4,4'-(1-methylethylidene)bis- /	$C_{15}H_{16}O_2$	228.2863	Not activity found
23.675	0.2689	*Bicyclo(7.2.0)undec-4-ene, 4,11,11-trimethyl-8-methylene-, (E)-(1R,9S)-(-)-/ β-Caryophyllene	$C_{15}H_{24}$	204.3511	Not activity found
26.619	2.088	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32	Antimalarial, Antifungal, Antioxidant bioactive, Anticancer, Antibacterial
31.072	0.1078	*Trehalose / α-D-glucopyranosyl-(1→1)-α-D-glucopyranoside	$C_{12}H_{22}O_{11}$	342.296	To treat dry eye
10.357	0.307	*Alpha. Terpinene	$C_{10}H_{16}$	136.23	Not activity found
19.668	0.3369	81-methoxy-4-(1-oropenyl)-benzene/ Anisole, p-propenyl-, Anethole	$C_{10}H_{12}O$	148.2	Anethole has potent antimicrobial properties

Cont. Table 3. Compositions detected in the methanol extract of Plantago lanceolata aerial organ by GC-MS analysis

26.204	0.2281	Tetradecamethylcycloheptasiloxane	$C_{14}H_{42}O_7Si_7$	519.07	Antifungal, Skin-Conditioning Agent, Fragrance, Antimicrobial, Antifouling, Imunomodulatory, Antitumor
28.415	0.6821	*Nonanoic acid/ Pelargonic acid	$C_9H_{18}O_2$	158.24	Not activity found
37.651	0.8193	*Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_1$	270.4507	Antioxidant, Nematicidal, Pesticidal, Hemolytic, Antiinflammatory, Cancer preventive, Hepatoprotective, Antihistaminic, Anticoronary, Antibacterial, Antifungal
42.328	0.6866	*Methyl stearate/ Methyl octadecanoate	$C_{19}H_{38}O_2$	298.5	Antifoaming, fermentation nutrient
41.733	0.7474	*9-Octadecenoic acid (Z)-, methyl ester/ Oleic acid methyl ester	$C_{19}H_{36}O_2$	296.5	Not activity found
41.584	0.3162	*9,12-Octadecadienoic acid (Z,Z)-, methyl ester/ Linoleic acid, methyl ester; Methyl linoleate	$C_{19}H_{34}O_2$	294.4721	Not activity found
45.761	0.3182	1-Octadecene/ alpha-Octadecene	$C_{18}H_{36}$	252.486	Not activity found
47.908	1.1833	Eicosane	$C_{20}H_{42}$	282.5	Antifungal, Antitumor, Antibacterial
12.642	1.3835	Tetrasiloxane, decamethyl-	$C_{10}H_{30}O_3Si_4$	310.6854	Lubricant additives, Personal Care additives
42.02	1.0141	*2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, (R-(R*,R*-(E)))- (CAS)/ Phytol	nd	nd	Not found activity
15.512	0.2744	*2H-1-benzopyran/ 2H-Chromene	C_9H_8O	132.16	No activity found
12.015	0.2011	*Benzofuran/ Coumarone	C_8H_6O	118.13	Not found activity
31.253	0.4773	Cyclododecane (cyclic compound)	$C_{12}H_{24}$	168.324	An intermediate in the production of flame retardants, Detergents, and other Chemicals
36.525	0.8919	Cyclotetradecane (cyclic hydrocarbon)	$C_{14}H_{28}$	196.37	Not found activity

Cont. Table 3. Compositions detected in the methanol extract of Plantago lanceolata aerial organ by GC-MS analysis

38.289	2.6725	*Palmitoleic acid/cis-9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254.41	Anti-inflammatory, Improves insulin
		(monounsaturated omega-7 fatty acid)			sensitivity in liver and skeletal
42.732	5.6608	*cis-Vaccenic acid/cis-11-Octadecenoic acid; Asclepic acid	$C_{18}H_{34}O_2$	282.5	Antibacterial, Hypolipidemic effect in rats
29.435	0.4044	Dihydroxymaleic acid/ Maleic acid, dihydroxy-	$C_4H_4O_6$	148.07	No activity found
23.366	0.5912	Dihydroxymaleic acid (different CAS Number)	nd	nd	No activity found
32.241	0.1739	D-erythro-Pentose, 2-deoxy-/ 2-Deoxy-D-ribose; Thyminose; 2-Deoxy-D-arabinose	$C_5H_{10}O_4$	134.1305	No activity found
10.251	0.3296	*Benzene, 1-methyl-3-(1-methylethyl)-/ 3-Isopropyltoluene; M-Cymene; m-Cymene; β-Cymene; m-Cymol	$C_{10}H_{14}$	134.22	No activity found
28.819	0.7027	Ethanol, 2-bromo-	C_2H_5BrO	124.96	No activity found
20.805	0.2357	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	444.9236	Antifungal, Emollient, Personal care products, Lubricant, de-foaming agent
14.927	0.3012	Cyclopentasiloxane, decamethyl-/ Cyclomethicone 5	$C_{10}H_30O_5Si_5$	370.77	Cosmetic and personal products as a skin
4.6174	1.7427	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222.462	Antimicrobial, Antioxidant activity
40.989	0.4281	*Myristic acid	$C_{14}H_{28}O_2$	228.37	Antimicrobial, Antispasmodic, Antiinflammatory
33.942	2.4264	Myristic acid (different CAS number)	nd	nd	No activity found
9.5279	0.58465	Cyclotetrasiloxane, octamethyl-/Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_4Si_4$	296.61	Antibacterial, Antiseptic, Hair conditioning agent, Skin conditioning agent- emollient
43.89	0.9599	*Octadecane/ n-Octadecane	$C_{18}H_{38}$	254.5	Not found activity
6.1586	0.7054	Benzoic acid, 2-methoxy-, methyl ester/ o-Anisic acid, methyl ester	$C_9H_{10}O_3$	166.17	A flavouring ingredien
41.35	0.7979	Cetene/ 1-Hexadecene	$C_{16}H_{32}$	224.4253	Not activity found

Cont. Table 3. Compositions detected in the methanol extract of Plantago lanceolata aerial organ by GC-MS analysis

40.829	1.8758	Benzene, 1-(bromomethyl)-3-chloro-	C_7H_6BrCl	205.48	No activity found
36.631	0.185	1H-Phosphole, 2,5-dihydro-1-methyl-/ 1-methyl-3-phospholene	C_5H_9P	100.1	No activity found
36.227	0.8002	*Pentadecanoic acid	$C_{15}H_{30}O_2$	242.4	Antiinflammatory, Antifibrotic, Red blood cell-Stabilizing, mitochondrial-reparative
3.4802	3.1108	Silanediol, dimethyl-	$C_2H_8O_2Si$	92.17	Aermatological disorder, Acne, Immunological disorder, viral infection
12.546	0.2766	*Benzene, 1-methyl-4-(1-methylethenyl)-/ p-Cymenene; Dehydro-p-cymene; 2-P-Tolylpropene; p-alpha-Dimethyl styrene; P-Mentha-1,3,5,8-tetraene	$C_{10}H_{12}$	132.2	A flavouring ingredien
35.844	0.1189	*Oxacyclododecan-2-one/ undecalactone	$C_{11}H_{20}O_2$	184.27	No activity found
25.875	0.2116	Oxirane, 2,3-dimethyl-, cis-	C_4H_8O	72.1057	Antifungal, Antibacterial activities
39.363	2.3109	Isoxazole, 3,5-diphenyl-/ 3,5-Diphenyl-isoxazole	$C_{15}H_{11}NO$	221.25	Not found activity
32.348	0.1196	*1,2,3,4-Butanetetrol, (S-(R*,R*))-/ l-Threitol	$C_4H_{10}O4$	122.12	Not found activity
46.93	0.3305	Estra-1,3,5(10)-trien-17.beta.-ol	$C_{18}H_{24}O$	256.3826	Antiarrhythmic, Antiosteoporosis
50.427	0.2237	Trihexadecyl borate	$C_{48}H_{99}BO_3$	735.1	Cosmetics, personal care products, as an emollient, skin conditioning agent.
13.131	0.1853	3(2H)-Furanone, dihydro-2-methyl-/ 2-Methyl tetrahydro-3-furanone; 2-Methyloxolan-3-one	$C_5H_8O_2$	100.12	As coffee furanone, in many foods
16.309	0.1044	5H-1,4-Dioxepin, 2,3-dihydro-/ 2,3-Dihydro-5H-1,4-dioxepine; 5H-1,4-Dioxepin, 2,3-dihydro-	$C_5H_8O_2$	100.12	As a building block in the synthesis of pharmaceuticals and agrochemicals
19.848	0.1919	Anisole, p-isopropyl-	$C_{10}H_{14}O$	150.22	Perfumes, flavorings, pharmaceuticals
19.434	2.1268	*Trans-anethole	$C_{10}H_{12}O$	148.2	Fungicides, Antioxidant, Antimicrobial, Antiplatelet, Oestrogenic agent

Cont. Table 3. Compositions detected in the methanol extract of Plantago lanceolata aerial organ by GC-MS analysis

34.091	0.3661	1,2,3-Trimethoxy-5-((1E)-2-nitro-1-propenyl)benzene/ 1,2,3-trimethoxy-5-(2-nitroprop-1-enyl)benzene	$C_{12}H_{15}NO_5$	253.25	Not found activity
35.143	0.1238	N,N-Dimethylethanesulfonamide/ N,N-Dimethylethanesulphonamide	$C_4H_{11}NO_2S$	137.2	Not found activity
47.982	0.1989	*1-Dotriacontanol	$C_{32}H_{66}O$	466.9	Antimicrobial
11.367	0.5458	1-Methoxy-4-(phenylethynyl)benzene/ 1-methoxy-4-(2-phenylethynyl)benzene	$C_{15}H_{12}O$	208.25	Not found activity
20.539	0.1686	*4-vinyl-2-methoxy-phenol/4-vinylguaiacol; p-Vinylguaiacol	$C_9H_{10}O2$	150.17	As a pheromone, a flavouring agent
52.181	0.863	Cyclopropanenonanoic acid, 2-((2-butylcyclopropyl)methyl)-, methyl ester	$C_{21}H_{38}O_2$	322.5	Not found activity
31.157	0.1764	Alpha.-D-Mannopyranoside, methyl 3,6-anhydro-	$C_7H_{12}O_5$	176.17	Not found activity
34.59	0.133	Ethyl 4-isothiocyanatobutyrate/Ethyl 4-isothiocyanatobutanoate; 4-Isothiocyanatobutanoic acid ethyl ester	$C_7H_{11}NO_2S$	173.24	Not found activity
34.271	0.3987	*1,2-Octadecanediol/ Stearyl glycol	$C_{18}H_{38}O_2$	286.5	Cosmetics, personal care products as an emollient and moisturizer
51.107	1.9686	*Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester/2-Palmitoylglycerol; 2-Monopalmitin; 1,3-dihydroxypropan-2-yl palmitate; 2-Monopalmitoylglycerol	$C_{19}H_{38}O_4$	330.5	Mono- and Diglycerides are commonly added to commercial food products in small quantities, Emulsifiers
47.472	0.4087	*16-Methyl-heptadecanecarboxylic acid/ Isooctadecanoic acid; Isostearic acid	$C_{18}H_{36}O_2$	284.5	Cosmetics, personal care products as an emollient, thickening agent.
37.045	0.3136	Benzonitrile, m-phenethyl-/1-(3-Cyanophenyl)-2-phenylethane	$C_{15}H_{13}N$	207.27	Not found activity
4.66	0.4012	1H-Isoindole-1,3(2H)-dithione, 2-ethyl-/ 2-Ethyl-1H-isoindole-1,3(2H)-dithione	$C_{10}H_9NS_2$	207.31516	Industrial applications,, production of dyes and pigments
39.501	1.2377	Ethyl (2E)-3-(4-Bromophenyl)-2-(Diethoxyphosphoryl)-2-Propenoate	$C_{15}H_{20}BrO_5P$	391.194	Pesticides
39.235	0.5351	Trichloroacetic acid, hexadecyl ester/Hexadecyl trichloroacetate	$C_{18}H_{33}Cl_3O_2$	387.8	Not found activity

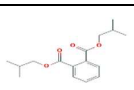
Cont. Table 3. Compositions detected in the methanol extract of Plantago lanceolata aerial organ by GC-MS analysis

39.926	0.459	3-Piperidinol, 1,4-dimethyl-, trans-/ trans-1,4-Dimethyl-3-piperidinol	$C_7H_{15}NO$	129.2	Industrial applications, production of pharmaceuticals, agrochemicals
13.205	0.2929	3-Piperidinol, 1,4-dimethyl-, cis-/ cis-1,4-Dimethyl-3-piperidinol	$C_7H_{15}NO$	129.2	Not found activity
16.394	0.1362	N-Nitroso-2-methyl-oxazolidine/2-Methyl-3-nitrosooxazolidine	$C_4H_8N_2O_2$	116.12	Not found activity
3.714	1.1475	2-(((Methylsulfonyl) methyl) sulfanyl) ethanol/3,5 -Dithiahexanol 5,5-dioxide	$C_4H_{10}O_3S_2$	170.3	Not found activity
37.545	0.1686	1H-Indole, 2,5-dimethyl-1-(trimethylsilyl)-/ 2,5-Dimethylindole, TMS derivative	$C_{13}H_{19}NSi$	217.38	Not found activity
13.641	0.1612	(2,2'-Bipyridine)-6,6'-diethanol, .beta.,.beta.'-bis(methylene)-	nd	nd	Not found activity
27.947	0.1795	Benzaldehyde, 2-nitro-, diaminomethylidenhydrazone	$C_8H_9N_5O_2$	207.19	No activity found
31.827	0.2666	Cyclohexanone, 2-hydroxy-4-methyl-2-(2-oxopropyl)-, (2S-cis)-	nd	nd	Not found activity
47.036	0.1326	Cyclohexane, 2-(dimethylhydrazono) -3-(4-hexenyl)-1-aci-nitro-, (E,E)-	$C_{14}H_{25}N_3O_2$	267.367	No activity found
29.924	0.3907	1H-Inden-1-one, 2,3-dihydro-6-hydroxy-2,2,7-trimethyl-4-(1-methylethyl)-/ 6-hydroxy-4-isopropyl-2,2,7-trimethylindan-1-one	$C_{15}H_{20}O_2$	232.32	No activity found
28.489	0.824	9,10-Anthracenediol, 1,4,4a,5,8,8a,9,9a,10,10a-decahydro-2,3,6,7-tetramethyl-, mono(4-methylbenzenesulfonate), (4a.alpha.,8a.beta.,9.beta.,9a.beta.,10 .alpha.,10a.alpha.)-	nd	nd	Not found activity
35.058	0.2946	2-(Trideuteromethyl)propanol	nd	nd	Not found activity
50.044	0.0843	(trans)-2-Azidocyclopentan-1-ol	nd	nd	Not found activity
3.2357	5.6636	*2,3-Dihydroxysuccinic acid/ Tartaric acid	$C_4H_6O_6$	150.087	Expectorant, Industrial use
20.635	0.0491	2-Methoxy-5-vinylphenol/5-ethenyl -2-methoxyphenol	$C_9H_{10}O_2$	150.174	Not found activity
8.2631	0.7015	Oxime-, methoxy-phenyl-	$C_8H_9NO_2$	151.16	Antifungal, Antibacterial, Anticancer, Antitumor
35.302	0.1324	*Styracitol/ 1,5-Anhydro-d-mannitol	$C_6H_{12}O_5$	164.16	Not found activity

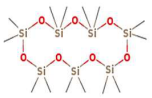
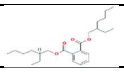
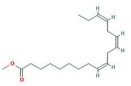
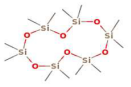
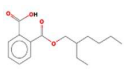
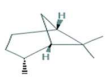
Cont. Table 3. Compositions detected in the methanol extract of Plantago lanceolata aerial organ by GC-MS analysis

29.18	0.3647	1-Methyl-2-(methylamino)-1-phenylethanol/ 1-(methylamino)-2-phenylpropan-2-ol	$C_{10}H_{15}NO$	165.23	Pharmaceuticals, industrial applications
35.472	0.3065	5-Phenyl-4,5-dihydro-1H-(1,2,4)triazin-6-one	nd	nd	Not found activity
28.213	0.5187	(R*,S*)-2-(1'-Nitroethyl)-2-methyl-1,3-oxathiolane	nd	nd	Not found activity
47.302	0.1252	Butanamine, 2,2-dinitro-N-methyl-/ N-(2,2-dinitrobutyl)-N-methylamine	$C_5H_{11}N_3O_4$	177.16	Not found activity
40.032	0.4581	Trans-1-Chlorocyano methyl-2-trifluoromethyl-cyclopropane/ Chloro(2- (trifluoromethyl) cyclopropyl)acetonitrile	$C_6H_5C_lF_3N$	183.56	Not found activity
36.939	1.1978	1-benzylindole	$C_{15}H_{13}N$	207.27	No activity found
32.539	0.0934	Methyl 9-Methylundecanoate	nd	nd	Not found activity
34.909	0.4094	*2-Heptadecenal/cetenal	$C_{17}H_{32}O$	252.4	Not found activity
41.871	0.3274	*Cis-9-Tetradecenoic acid, propyl ester/ Oleic acid propyl ester; n-propyl elaidate	$C_{21}H_{40}O_2$	324.5	Not found activity
40.223	1.3684	1H-Isoindole-4-carboxylic acid, 2-((2-fluorophenyl)methyl)-2,3-dihydro-3-oxo-/ 2-((2-fluorophenyl)methyl)-3-oxoisoindoline-4-carboxylic acid	$C_{16}H_{12}FNO_3$	285.27	Not found activity
33.304	0.1353	Adenosine, 4'-de(hydroxymethyl)-4'-(N-ethylaminoformyl)-	$C_{22}H_{48}O_9Si$	484.6966	Not found activity
27.809	0.1074	Octaethylene glycol, TBDMS derivative	$C_{22}H_{48}O_9Si$	484.6966	Not found activity

Table 3. Compositions detected in the methanol extract of Plantago lanceolata aerial organ by GC-MS analysis

RT	Area Pct	Library/ID	Formula	M.w (g/mol)	Biological activity	Structure
38.8721	1.5312	n-Hexadecanoic acid/ Palmitic acid	$C_{16}H_{32}O_2$	256.42	Not found activity	
42.7974	1.1562	*9,12-Octadecadienoic acid (Z,Z)-/ Linoleic acid	$C_{18}H_{32}O_2$	280.4455	Antimicribial, Antifungal, Hepatoprotective, Antihistaminic, Antiinflammatory, Cancer preventive, Antiandrogenic, Nematicide, Antioxidant	
44.2965	0.1877	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester/ Diisobutyl phthalate;	$C_{16}H_{22}O_4$	278.3435	Antimicrobial, α-Glucosidase inhibition, *in vivo* hypoglycemic	

Cont. Table 4. Compositions detected in the acetonic extract of Plantago lanceolata Aerial organ by GC-MS analysis

26.2151	0.2687	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	519.07	Antiperspirants, Antibacterial, Antifungal, Antimicrobial, Antiseptic, Hair Conditioning Agent, Skin- Conditioning Agent-Emollient	
37.6819	0.1653	*Hexadecanoic acid, methyl ester/ Methyl palmitate	$C_{17}H_{34}O_2$	270.5	Antioxidant, Nematicidal, Pesticidal, Hemolytic, Antiinflammatory, Cancer preventive, Hepatoprotective, Antihistaminic, Anticoronary, Antibacterial, Antifungal	
42.9003	1.3408	*Oleic Acid/ cis-9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5	Antioxidant, Emulsifying, Emollient, An excipient in pharmaceuticals	
50.8138	1.0958	Bis(2-ethylhexyl) phthalate/ Di(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.6	Antibacterial, Antifungal, Cytotoxic	
41.7674	0.1862	*9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-/ Methyl linolenate	$C_{19}H_{32}O_2$	292.5	An insect attractant, Anticancer, Antibacterial, Antioxidant, Antipyretic, Cardioprotective, Neural function, Antiandrogenic (5-alpha reductase inhibitor), Antiarthritic	
35.6678	0.116	*2-Pentadecanone, 6,10,14-trimethyl-/ Hexahydrofarnesyl	$C_{18}H_{36}O$	268.5	Not activity found	
20.8307	0.2247	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	444.92	Emollient, Personal care products, Lubricant, de-foaming, Antifungal, Antioxidant, Antifungal,	
5.1067	0.1523	*2-Hexanol, 2-methyl-	$C_7H_{16}O$	116.2	Not activity found	
38.6089	0.1105	*Hexadecenoic acid, Z-11-	$C_{16}H_{30}O_2$	254.41	Not activity found	
52.765	92.602	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester/ Mono (2-ethylhexyl) phthalate	$C_{16}H_{22}O_4$	278.3435	Antimicrobial, Cytotoxicity, Antioxidant, Antiinflammatory, Antiviral	
35.4847	0.4714	*Bicyclo(3.1.1)heptane, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5. alpha.)-/ cis-Pinane	$C_{10}H_{18}$	138.25	Not activity found	

Cont. Table 4. Compositions detected in the acetonic extract of Plantago lanceolata Aerial organ by GC-MS analysis

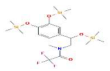
31.0787	0.0829	N-(Trifluoracetyl)-O,O',O''-tris(trimethylsilyl) epinephrine	$C_{20}H_{36}F_3NO_4Si_3$	495.8	Not activity found	
41.6243	0.0951	*10,13-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.5	Anti-inflammatory, Anti-arthritic, Hypocholesterolemic, Hepatoprotective, Antihistamine activity	
36.5661	0.213	*3,7,11,15-Tetramethyl-2-hexadecen-1-ol/ Phytol	$C_{20}H_{40}O$	296.5	A precursor for the manufacture of synthetic forms of vitamin E and vitamin K_1.	

Table 4. Compositions detected in the acetonic extract of Plantago lanceolata Aerial organ by GC-MS analysis

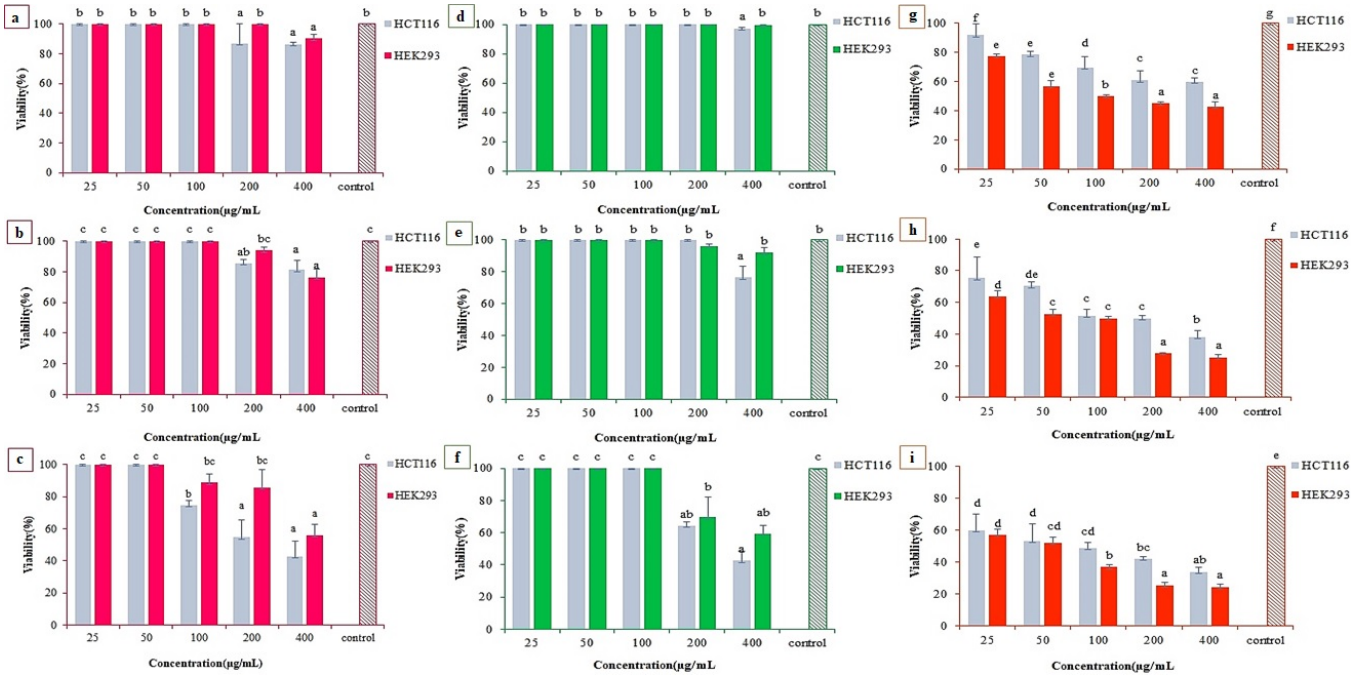


Fig. 1. Cytotoxicity assay of P. lanceolata extracts by MTT method against the colorectal carcinoma cell lines (HCT-116) and embryonic kidney normal cell line (HEK-293) at 24, 48, and 72 h, respectively. The methanolic extract (a, b, and c), ethanolic extract (d, e, and f), and acetonic extract (g, h, and i). Values represent the mean of three replications ± standard deviation. Duncan test was used for mean comparison ($p<0.05$). Different letters above the column indicate significant differences among different concentrations)

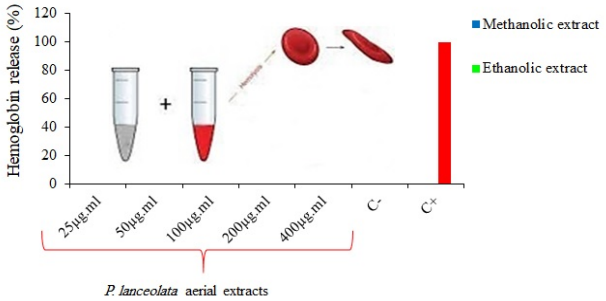


Fig. 2. Hemolysis assay to verify the biocompatibility of methanolic and ethanolic extracts of P. lanceolata aerial. Positive control (C^+) and negative control (C^-)

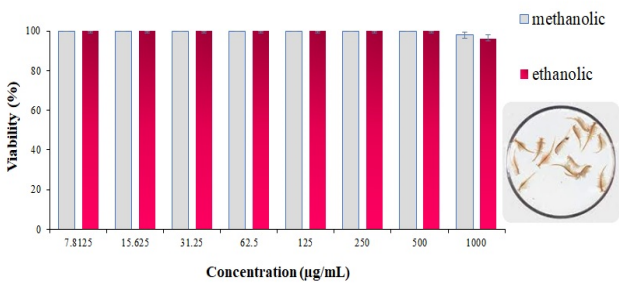


Figure 3. Cytotoxic Activity of P. lanceolata Aerial extracts on A. Salina

DISCUSSION

Our investigation began the synthesis of alcoholic and acetonic extracts of P. lanceolata aerial organs, guaranteeing the extraction of the bioactive metabolites. The current study was executed to evaluate the cytotoxic effects of P. lanceolata extracts using MTT procedure. The present study's finding confirmed that acetonic aerial extract of P. lanceolata exhibited a good antiproliferative report in the range of 10% to 67% against colon cancer cell line (HCT-116) at 25 to 400µg/mL. However, P. lanceolata acetonic extract was also cytotoxic against HEK-293 Human Embryonic Kidney cells (23% to 76%) which limited its medicinal use due to its high toxicity on normal cells. The roots of P. major and P. lanceolata were acetonic extracted, and the IC50 values for each have been calculated.

The study found that P. lanceolata acetonic root extract had a higher IC50 (119.68 µg/mL) against HCT-116 than P. major root acetonic extract (82µg/mL) (19, 25). Regarding HCT-116, the IC50 values of P. major's methanolic, ethanolic, and acetonic aerial extracts were 655.09µg/mL, 475.20µg/mL, and 221.64µg/mL, respectively (19). Nevertheless, compared to P. major extracts, the IC50s for the methanolic, ethanolic, and acetonic extracts of P. lanceolata aerial organs were lower. Additionally, it was observed that the essential oil extracted from P. major had a more pronounced cytotoxic effect on HCT-116 cells (IC50=158.33µg/mL) than P. lanceolata essential oil (IC50=102.66 µg/mL) (26). A separate research assessed the cytotoxic potential of P. lanceolata root extracts in butanol, ethyl acetate, and dichloromethane at varying doses. The IC50 values of P. lanceolata root extracts in dichloromethane and ethyl acetate (167.458 µg/mL and 168.553 µg/mL) against HCT-116 were found to be lower than those of butanol extract (205.004 µg/mL) (27). Similar studies were reported by other researchers also corroborating our findings. It has been demonstrated that the methanolic extract of P. major has antiproliferative properties against the HCT-15 colon cancer cell line (28). A different research examined the cytotoxic effects of P. lanceolata extracts on a range of cell lines, including as the National Cancer Institute's Human kidney adenocarcinoma TK-10, human melanoma cells UACC-62, and breast cancer MCF-7 cells. Additionally, HT-29 colon adenocarcinoma, MCF-7 breast adenocarcinoma, HeLa cervix epitheloid carcinoma, and human fetal lung (MRC-5) malignancies were reported to be sensitive to P. lanceolata aerial extracts. The researchers found that several bioactive compounds, including luteolin-7-O-glucoside, apigenin, gallic acid, and vanillic, had strong cytotoxic effects (13). Furthermore, gallic acid and apigenin were found to be present in a large number of P. major and P. lanceolata organs (29, 30, 31, 32). After that, another researcher reported that MCF-7 cell proliferation was reduced by treating P. lanceolata leaf aqueous extract (33). On the other hand, P. lanceolata leaf extract showed a significantly reduced proliferation of CAL-51 triple-negative breast cancer cells, but minor cytotoxic activity was observed against AMJ-13, MCF-7, and MDAMB breast cancer cell lines (34). In 2018, it was demonstrated that P. lanceolata extracts might activate the prostate cancer cell lines Du-145 and PC-3 (35). P. major and P. lanceolata root methanolic extracts had IC50 values of 470µg/mL and 282.94µg/mL against HCT-116 at 72 hours in our earlier research. However, the extracts' IC50 values against HEK-293 were determined to be 1563µg/mL and 962.38 µg/mL, respectively (19, 25).

The alcoholic extracts exhibited biocompatibility effects using a hemolysis assay. Prior to this, the biocompatibility of alcoholic extracts from P. major and P. lanceolata root's aerial and root organs was also investigated. At 400 µg/mL concentration, the level of hemolysis in P. lanceolata and P. major extracts was less than 1%. (19, 25). This is similar to alcoholic extracts derived from P. lanceolata aerial organs.

If an extract's LC50 value is more than 1000 µg/mL, it is generally regarded as non-toxic (36). In our earlier research, we found that treating the P. lanceolata root methanolic extract at 1000 µg/mL resulted in a mortality (%) of less than 3% for all nauplii, with an LC50:27.25 mg/mL (25). The assessment of the overall toxicity of P. major and P. lanceolata essential oils revealed no harmful effects (LC50: 1783.7 µg/mL and 2242.57 µg/mL)(26). Testing against A. Salina indicated that the ethanolic extracts from P. major's aerial and root organs were non-toxic. (19). However, The toxicity of P. major methanolic extract was also evaluated against A. uramiana and it exhibited an LC50 value of 303.7 µg/mL (37). Plantago squarrosa Murray extracts showed non-toxic on the Artemia franciscana as LC50 values of the extracts exceeded 1000 µg/mL (38). Caro et al. (2018) observed that aqueous crude extract derived from the leaves of P. major causes no mortality or macroscopic signs of toxicity at 1000 mg/Kg (34). Similarly, the alcoholic extracts of aerial and root organs of P. major did not induce acute toxicity at a dose of 2000 mg/kg (19). Additionally, the aqueous and alcoholic extracts of P. lanceolata leaf and root caused no toxicity at 2000 mg/kg (25, 40). During this study, the animals did not exhibit any significant weight changes, indicating their normal behavior. As a result, changes in body weight serve as a crucial index for detecting the toxicity of a formulation.

By conducting a comparison of previous studies

focused on P. lanceolata root (25), It was discovered that there were common components (%) in both methanolic extracts of the plant's aerial and root organs. These components include n-Hexadecanoic acid (15% and 10.68%); Octadecanoic acid (9.8% and 4.90%); 9,12-Octadecadienoic acid (Z,Z)- (0.95% and 3.04%); and 1-Dotriacontanol (0.19% and 1.12%). Dihydroxymaleic acid (0.40% and 1.29%); Cyclohexasiloxane, dodecamethyl- (0.23% and 0.82%); Cyclotetrasiloxane, octamethyl- (0.54% and 0.81%); and (trans)-2-Azidocyclopentan-1-ol (0.08% and 0.46%) were also found in result of GC-MS of both methanolic extracts of the plant's aerial and root organs. However, these components are not from the plant's original. The main ingredients in the P. lanceolata root acetonic extract were 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester (72.60%) and bis(2-ethylhexyl) phthalate (21.04%) (20). These ingredients were found in the acetonic extract of P. lanceolata's aerial organ at 1.09% and 92.60%, respectively. In the P. lanceolata acetonic root extract, diisooctyl phthalate (3.90%) was also found. In the present investigation, the acetonic extract of P. lanceolata aerial organs also included diisobutyl phthalate (0.18%). P. major's leaf and root organs had varying percentages of dioctyl phthalate, ranging from around 3.26 to 80.55% and 2.52 to 19.69%, respectively (41). As a means of eliminating environmental contaminants, the researchers came to the conclusion that the potential to accumulate diethyl and octyl phthalate may be highly present in the leaves and roots of P. major. This issue can also be confirmed in the case of the production of phthalate in acetonic extracts of P. lanceolata. Consequently, plants can produce dioctyl phthalate and diethyl phthalate, which can serve as plant phytotoxins. Cycloheptasiloxane, tetradecamethyl- (0.26% and 0.10), and Cyclohexasiloxane, dodecamethyl- (0.22% and 2.11) were also found in both acetonic extracts of aerial and root organs of P. lanceolata. Siloxanes may be present due to contamination, column bleed, or other factors, and are not from the extract itself but come from the GC column. It is important to note that siloxanes, phthalates, and halogenated compounds are unlikely to occur naturally (42). The primary constituents of P. lanceolata leaf essential oil were discovered to be fatty acids, including linoleic, linolenic, myristic, and palmitic acids, according to the examination of the oil (43). In the current investigation, P. lanceolata methanolic and acetonic extracts showed varying amounts of linoleic acid (0.31% and 1.15%) and palmitic acid (15.00% and 1.53%), respectively. The methanolic extract of P. lanceolata aerial organs also contained myristic acid. These elements were also observed in P. lanceolata methanolic root extracts. (25). The anti-cancer properties may be attributed to the presence of (44, 45), n-hexadecanoic acid, 9, 12-octadecadienoic acid (Z, Z)- (linoleic acid) (46); Hexadecanoic acid, methyl ester (47); Benzofuran (48); 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-(Methyl linolenate) (49).

CONCLUSION

The methanolic and ethanolic extracts did not induce hemolysis and were more cytotoxic against HCT-116 compared to HEK293. From a pharmaceutical point of view, if toxic drugs show selective toxicity on cancer cells and are non-toxic on normal cells, it is considered advantageous (therapeutic index). Therefore, alcoholic extracts can be a better option in suppressing the growth of colorectal cancer cells in vitro compared to acetonic extracts as acetonic extracts demonstrated notable cytotoxic effects in both normal and cancer cells. However, determining the bioactivity of extracts requires investigating their chemical composition to ascertain whether it is influenced by a single metabolite or a combination of multiple metabolites.

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