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Polyphenol Constituents of Different Extract of *Lepidium Sativum* Seed by High Performance Liquid Chromatography (HPLC) Against Pathogenic Microorganism

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ABSTRACT

Lepidium sativum Polyphenol constituents of (aqueous, ethyl acetate and petroleum ether) seed extracts were examined by HPLC, the aqueous extract contained 17 compounds were 3,4,5-trihydroxybenzoic acid (gallic acid), 3(3,4-Dihydroxycinnamoyl)quinate (Chlorogenic acid), (2R,3S)-2-(3,4Dihydroxphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol (Catechin), 1,3,7-Trimethylpurine-2,6-dione (caffeine), 3,4-Dihydroxycinnamic acid (Coffeic acid), 4-Hydroxy-3,5-dimethoxy benzoic acid (Syringic acid), Rutin, Benzene-1,2-diol (Pyro catechol), 4,4,5,5,6,6-Hexahydroxydiphenic acid 2,6,2,6-dilactone (Ellagic acid), 4-Hydroxycinnamic (Coumaric acid), 4-Hydroxy-3-methoxy benzaldehyde (Vanillin), hydroxycinnamic acid (Ferulic acid), 5,7-Dihydroxy-2-(4-hydroxyphenyl)chroman-4-one(Naringenin), Proply 3,4.5-trihydroxybenzoate(PropylGallate),4`.7-DihdroxyisoFlavone, 2-3,4dihyoxyphenyl-3.5.7-trihydroxy-4H-chromen-4-one,(Quercetin), (2E)-3-phenylprop-2-enoic acid (Cinnamic Acid). The *Lepidium sativum* ethyl acetate and petroleum ether seed extract contained 13 compounds were 3,4,5-trihydroxy benzoic acid (gallic acid), 3(3,4-Dihydroxycinnamoyl)quinate (Chlorogenic acid), (2R,3S)-2-(3,4Dihydroxphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol(Catechin),1,3,7-Trimethylpurine-2,6dione(caffeine), 4-Hydroxy-3,5-dimethoxybenzoic acid (Syringic acid), Rutin, 4,4,5,5,6,6-Hexahydroxydiphenic acid 2,6,2,6-dilactone (Ellagic acid),4-Hydroxycinnamic (Coumaric acid), 4-Hydroxy-3-methoxy benzaldehyde (Vanillin), hydroxycinnamic acid (Ferulic acid), 5,7-Dihydroxy-2-(4-hydroxyphenyl)chromn-4-one (Naringenin),2-(3,4-dihyoxyphenyl-3.5.7-trihydroxy-4H-chromen-4-one,(Quercetin) (2E)-3-phenylprop-2-enoic acid (Cinnamic Acid). The four type of different pathogenic bacteria (*Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Bacillus cereus0*, treated with different seed extract of *Lepidium sativum* (Aqueous, ethyl acetate, petroleum ether) by Mueller Hinton Agar and measuring inhibition zone (diameter mm), show that there were significant differences among bacteria and different method of extract. All different *Lepidium sativum* seed extract (aqueous, ethyl acetate, and petroleum ether) have high activity against *Candida albicans* fungus. The study was conducted to identify the *Lepidium sativum* polyphenol Compound and the activity against bacteria and fungi.

Introduction

Lepidium sativum locally identified garden cress, garden pepper cress, peppergrass, pepperwort, ElRashad the family Brassicaceae (Cruciferae). The Origen of the plant is Ethiopia and distributed around all the world Asia, Mediteean[(1,2,3).] Western Europe and plant consist of carbohydrate, fatty acid volatile oils, protein, water-soluble vitamin C and vitamin B-complex, polyphenol[(4,5)]. The seeds of Lepidium. sativum is a diuretic. The plant contains flavonoid and have biological benefits[(6).] They have been boiled with milk and are used in the treatment of bacterial and fungal infections and prevent oxidation [(7,8,9)]. It used in rheumatic joints as pest to reduce the pain and swelling. It prevents the body against skin disease, dysentery, and diarrhea[(7)] Leaves, flowers, root seed are used in folk medicine. Leaves are used by Europeans as salads in scorbutic disease. oil extracted from the seed is also useful. Seeds are used in the skeletal system, as a good for healing of bone fraction, diuretic, toxic, alterative, aphrodisiac, carminative, galactagogue, anti-asthmatic [(10)]. *Lepidium* sativum seed extract contains phenolic compounds containing alkaloids, cardiac glycosides, anthraquinones glycosides, tannins, steroids, flavonoids[(11,12)]. The plant has potent activity against microorganisms and control parasites [(13,14).]

Methods

Material

Lepidium sativum seed purchase in herbiest in the super market and identified in the Faculty of Agriculture Department of Botany Khartoum University.

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Microorganism

The microorganisms used in this work were obtained from laboratories of Microbiology, Faculty of Agriculture Khartoum Sudan. The methods of bacteria identification was conventional biochemical methods[15] according to the standard microbiology techniques. These microbes were, *Pseudomona aeruginosa*, *Escherichia coli*, *Bacillus cereus* and *Salmonella typhimurium.*

Methods

Preparation of extracts

Weighted 100gm *Lepidium* sativum seeds powder and then subjected to different extraction solvents separately. Extracted by distilled water overnight at room temperature (25-30°C) filtered and dried, extracted by ethyl acetate 90% at 50°C-60°C for 2 h, petroleum ether 90% at 50°C-60°C for 2 h, in a Sox let apparatus. All solvents extract were evaporated by a Buchi Rotary evaporator under reduced pressure and dried using a rotary evaporator at 55°C. Dried extracts were stored in the refrigerator until used.

HPLC conditions

High Performance Liquid Chromatography (HPLC) (Shimadzu corporation (Koyoto analysis was carried out using an Agilent 1260 series. Using Kromasil C18 column (4.6 mm x 250 mm i.d., 5 µm), the separation temperature 35°C The mobile phase contains water (A) and 0.02% tri-floro-acetic acid in acetonitrile (B) at a flow rate of 1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (80% A); 5–8 min (40% A); 8-12 min (50% A); 12-14 min (80% A); 14-16 min (80% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 10 µl for each of the sample solutions.

Mueller Hinton Agar

The test culture was inoculated in Sterile Mueller Hinton agar media (Mueller Hinton Agar (Becton Dickinson M. D USA), media was prepared due to the manufacturer's instruction) using sterile wire loops in surface media then the entire surface of the plate spread bacterium to obtain uniformity of the inoculum. Concentrations of 12.5, 25, 50, and 100mg/ml prepared from the seed different extract (aqueous, ethyl acetate, and petroleum ether) were used as anti-pathogenic bacteria. Prepared the Plates of Mueller Hinton agar then taken to solidify on Petri dishes. Seeded the plate with a test bacterium. In each plate made four holes with a sterile 2.0 mm diameter cork borers. a given concentration of the extract mixed with plane sterile agar filled each of the four holes. At 37°c for 24 hours the plates were then incubated. A meter rule using to measure the diameters of zones of inhibition and the mean value for each organism was recorded [16]

Preparation of the fungal organism

The fungal is culture as fallow at temperature 25ºC for 4 days put in Peptone water,. The growth mat of fungal was harvested and washed by sterile normal saline and then suspended in it then stored in the refrigerator till used [17].

Statistical analysis

It was done according to Duncan, Multiple Range Test [17]

Results and discussion

The Polyphenol constituents of *Lepidium sativum* aqueous seed extract in table 1, figure I contain 17 compounds 3,4,5-trihydroxy benzoic acid (Gallic acid), **(**6.2878**%),** 3(3,4-Dihydroxycinnamoyl) quinate (Chlorogenic acid) (2.7322%), (2R,3S)-2-(3,4Dihydroxphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol (Catechin) (2.6834%), 1,3,7-Trimethylpurine-2,6-dione (caffeine) (0.7541%), 3,4-Dihydroxycinnamic acid (Coffeic acid) (0.8340%), 4-Hydroxy-3,5-dimethoxybenzoic acid (Syringic acid) (18.0151%), Rutin (0.0000%), Benzene-1,2-diol (Pyro catechol) (0.0000%), 4,4,5,5,6,6-Hexahydroxydiphenic acid 2,6,2,6-dilactone (Ellagic acid),

Table 1. Polyphenol constituents of *Lepidium sativum* aqueous seed extract identified by HPLC

No	Name of the Poly phenol compound	Ret. Time	Area%	Chemical formula
1	3,4,5-trihydroxybenzoic acid (Gallic acid)	3.115	6.2878	$C_7H_6O_5$
2	3(3,4-Dihydroxycinnamoyl)quinate (Chlorogenic acid)	3.514	2.7322	$C_{16}H_{18}O_9$
3	(2R,3S)-2-(3,4Dihydroxphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol ((Catechin)	3.835	2.6834	$C_{15}H_{14}O_6$
4	1,3,7-Trimethylpurine-2,6-dione (caffeine)	4.045	0.7541	$C_8H_{10}N_4O_2$
5	3,4-Dihydroxycinnamic acid (Coffeic acid))	4.860	0.8340	$C_9H_8O_4$
6	4-Hydroxy-3,5-dimethoxybenzoic acid (Syringic acid)	5.191	18.0151	$C_9H_{10}O_5$
7	Rutin	5.519	0.0000	$C_{27}H_{30}O_{16}$
8	Benzene-1,2-diol (Pyro catechol)	5.766	0.0000	$C_6H_6O_2$
9	4,4,5,5,6,6-Hexahydroxydiphenic acid 2,6,2,6-dilactone (Ellagic acid),	6.752	0.5060	$C_{14}H_6O_8$
10	4-Hydroxycinnamic (Coumaric acid)	7.751	0.2882	$C_9H_8O_3$
11	4-Hydroxy-3-methoxybenzaldehyde (Vanillin)	8.315	0.2594	$C_8H_8O_3$
12	hydroxycinnamic acid (Ferulic acid)	8.742	0.4164	$C_{10}H_{10}O_4$
13	5,7-Dihydroxy-2-(4-hydroxyphenyl)chromn-4-one (Naringenin	9.365	2.2033	$C_{15}H_{12}O_5$
14	5,7-Dihydroxy-2-(4-hydroxy-2-(4-trihydroxybenzoa, Propyl 3,4,5-trihydroxybenzoate (Propyl Gallate)	10.283	0.7304	$C_{10}H_{12}O_5$
15	4`.7-DihydroxyisoFlavone	10.409	0.9674	C15H10O4
16	2-(3,4-dihyoxyphenyl-3.5.7-trihydroxy-4H-chromen-4-one, (Querectin)	10.614	0.5271	$C_{15}H_{10}O_7$
17	(2E)-3-phenylprop-2-enoic acid (Cinnamic Acid)	11.165	0.9922	$C_9H_8O_2$

Table 2. Polyphenol constituents of Lepidium sativum ethyl acetate seed extract identified by HPLC

No	Name of the Poly phenol compound	Ret.Time	Area%	Chemical formula
1.	3,4,5-trihydroxybenzoic acid (Gallic acid)	3.305	3.9301	$C_7H_6O_5$
2.	3(3,4-Dihydroxycinnamoyl)quinate (Chlorogenic acid)	4.070	0.9963	$C_{16}H_{18}O_9$
3.	(2R,3S)-2-(3,4Dihydroxphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol (Catechin)	4.382	0.6246	$C_{15}H_{14}O_6$
4.	3,4-Dihydroxycinnamic acid (Coffeic acid)	6.043	3.4638	$C_9H_8O_4$
5.	4-Hydroxy-3,5-dimethoxybenzoic acid (Syringic acid)	6.456	2.4283	$C_9H_{10}O_5$
6.	Rutin	7.503	0.4373	$C_{27}H_{30}O_{16}$
7.	4,4,5,5,6,6-Hexahydroxydiphenic acid 2,6,2,6-dilactone (Ellagic acid),	9.059	0.5358	$C_{14}H_6O_8$
8.	4-Hydroxycinnamic (Coumaric acid)	9.297	0.7174	$C_9H_8O_3$
9.	4-Hydroxy-3-methoxybenzaldehyde (Vanillin)	9.849	11.7509	$C_8H_8O_3$
10	hydroxycinnamic acid (Ferulic acid)	10.034	7.1691	$C_{10}H_{10}O_4$
11	5,7-Dihydroxy-2-(4-hydroxyphenyl)chromn-4-one (Naringenin)	10.158	2.2211	$C_{15}H_{12}O_5$
12	2-(3,4-dihyoxyphenyl-3.5.7-trihydroxy-4H-chromen-4-one, (Querectin)	12.423	1.1065	$C_{15}H_{10}O_7$
13	(2E)-3-phenylprop-2-enoic acid (Cinnamic Acid)	14.307	0.6564	$C_9H_8O_2$

Table 3. Polyphenol constituents of Lepidium sativum petroleum ether seed extractidentified by HPLC

No	Name of the Poly phenol compound	Ret.Time	Area%	Chemical formula
1	3,4,5-trihydroxybenzoic acid (Gallic acid)	3.309	30.4811	$C_7H_6O_5$
2	3(3,4-Dihydroxycinnamoyl)quinate (Chlorogenic acid)	4.036	2.2133	$C_{16}H_{18}O_9$
3	(2R,3S)-2-(3,4Dihydroxphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol (Catechin)	4.599	3.1020	$C_{15}H_{14}O_6$
4	3,4-Dihydroxycinnamic acid (Coffeic acid)	5.986	13.8247	$C_9H_8O_4$
5	4-Hydroxy-3,5-dimethoxybenzoic acid (Syringic acid)	6.430	0.0000	$C_9H_{10}O_5$
6	Rutin	7.533	0.4765	$C_{27}H_{30}O_{16}$
7	4,4,5,5,6,6-Hexahydroxydiphenic acid 2,6,2,6-dilactone (Ellagic acid),	8.896	0.4489	$C_{14}H_6O_8$
8	4-Hydroxycinnamic (Coumaric acid)	9.543	0.4299	$C_9H_8O_3$
9	4-Hydroxy-3-methoxybenzaldehyde (Vanillin)	9.857	0.6746	$C_8H_8O_3$
10	hydroxycinnamic acid (Ferulic acid)	10.044	2.8698	$C_{10}H_{10}O_4$
11	5,7-Dihydroxy-2-(4-hydroxyphenyl)chromn-4-one (Naringenin)	10.379	1.1107	$C_{15}H_{12}O_5$
12	2-(3,4-dihyoxyphenyl-3.5.7-trihydroxy-4H-chromen-4-one, (Querectin)	12.642	3.2456	$C_{15}H_{10}O_7$
13	(2E)-3-phenylprop-2-enoic acid (Cinnamic Acid)	14.303	3.0273	$C_9H_8O_2$

(0.5060%), 4-Hydroxycinnamic (Coumaric acid) (0.2882%), 4-Hydroxy-3-methoxybenzaldehyde (Vanillin) (0.2594%), hydroxycinnamic acid (Ferulic acid),(0.4164%), 5,7-Dihydroxy-2-(4-hydroxyphenyl)chromn-4-one (Naringenin) (2.2033%), 5,7-Dihydroxy-2-(4-hydroxy-2-(4-trihydroxyben zoa, Propyl 3,4,5-trihydroxybenzoate (Propyl Gallate) (0.7304%), 4`.7-Dihydroxyisoflavone(0.9674%), 2-(3,4-dihy oxyphenyl-3.5.7-trihydroxy-4H-chromen-4-one, (Querectin) (0.5271%), (2E)-3-phenylprop-2-enoic acid (Cinnamic Acid), (Cinnamic Acid) (0.9922%). Table 2 and figure 2 show that the *Lepidium sativum* seed extracted by ethyl acetate contained 13 compounds 3,4,5-trihydroxybenzoic acid (Gallic acid) (3.9301**%),** 3(3,4-Dihydroxy cinnamoyl) quinate (Chlorogenic acid) (0.9963%), 2R,3S)-2-(3,4Dihydroxphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol (Catechin) (0.6246%), 3,4-Dihydroxycinnamic acid (Coffeic acid) (3.4638%),4-Hydroxy-3,5-dimethoxybenzoic acid (Syringic acid) (2.4283%), Rutin (0.4373%),4,4,5,5,6,6-Hexahydroxydiphenic acid 2,6,2,6-dilactone (Ellagic acid), (0.5358%), 4-Hydroxycinnamic (Coumaric acid) (0.7174%), 4-Hydroxy-3-methoxybenzaldehyde (Vanillin) (11.7509%), hydroxycinnamic acid (Ferulic acid) (7.1691%). 5,7-Dihydroxy-2-(4-hydroxyphenyl)chromn-4-one (Naringenin) (2.2211%), 2-(3,4-dihyoxyphenyl-3.5.7-trihydroxy-4H-chromen-4-one, (Querectin) (1.1065%), (2E)-3-phenylprop-2-enoic acid (Cinnamic Acid) (0.6564%). Table 3 and figure3 show that the *Lepidium sativum* seed extracted by petroleum ether contained 13 compounds 3,4,5-trihydroxybenzoic acid (Gallic acid) (30.4811%)**,** 3(3,4-Dihydroxycinnamoyl)quinate (Chlorogenic acid) (2.2133%), 2R, 3S)-2-(3,4Dihydroxphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol (Catechin) (3.1020%). 3,4-Dihydroxycinnamic acid (Coffeic acid) (13.8247%), 4-Hydroxy-3,5-dimethoxybenzoic acid (Syringic acid) (0.000%), Rutin (0.4765%), 4,4,5,5,6,6-Hexahydroxydiphenic acid 2,6,2,6-dilactone (Ellagic acid), (0.4489%), 4-Hydroxycinnamic (Coumaric acid) (0.4299%), 4-Hydroxy-3-methoxybenzaldehyde (Vanillin) (0.6746%), hydroxycinnamic acid (Ferulic acid) (2.8698%), 5,7-Dihydroxy-2-(4-hydroxyphenyl)chromn-4-one (Naringenin) (1.1107%), 2-(3,4-dihyoxyphenyl-3.5.7-trihydroxy-4H-chromen-4-one, (Querectin) (3.2456%), (2E)-3-phenylprop-2-enoic acid (Cinnamic Acid) (3.0273%), In figure 4 found the chemical structures of polyphenols. Many studies reported that the flavonoids, phenolic compounds, alkaloids, cardiac glycosides, anthroquinones glycosides, tannins and steroids prevent body against oxidation, cancer and inflammation[18,19,20]. The antibacterial activity of the *Lepidium sativum* against four different pathogenic bacteria *Escherichia coli*, *Pseudomona aeruginosa*, *Salmonella typhimurium* and *Bacillus cereus* and one fungus Candida by different method (Aqueous, ethyl acetate, petroleum ether) seed extract (the lowest concentration of the *Lepidium sativum* seed extract is (12.5 mg/ml) and the highest one is (100 mg/ml), Table 4 mention that the aqueous extract of *Lepidium sativum* seed, have no inhibition zone against *Bacillus cereus* but the highest inhibition zone was detected against *Escherichia coli* (16.75)**.** Table 5 show that the *Lepidium sativum* seed extracted by ethyl acetate, the high inhibited zone against *Salmonella typhimurium* and *Bacillus cereus* (13.75). Table 6 show the activity of the *Lepidium sativum* seed extracted by petroleum ether the highest activity against *Escherichia coli* (14.5) and have no inhibition zone against *Bacillus cereus*.

Table 4. Inhibition zone (in mm) for different concentrations of *Lepidium sativum* aqueous seed extract

Microorganism	Concentration of the Lepidium sativum seed extract by ethyl acetate				Mean microorganism
	12.5	25	50	100	
Escherichia coli	15	16	18	18	16.75
Pseudomonas aeruginosa	13	15	15	15	14.5
Salmonella typhimurium	12	12	16	16	14
Bacillus cereus	-	-	-	-	-
Candida albicans	14	15	15	20	16
Mean aqueous Lepidium sativum seed extract	10.8	11.6	12.8	13.8	

Table 5. Inhibition zone (in mm) for different concentrations of *Lepidium sativum* ethyl acetate seed extract

Microorganism	Concentration of the Lepidium sativum seed extract by ethyl acetate				Mean microorganism
	12.5	25	50	100	
Escherichia coli	12	12	15	15	13.5
Pseudomonas aeruginosa	12	12	13	15	13
Salmonella typhimurium	12	12	14	17	13.75
Bacillus cereus	12	12	15	16	13.75
Candida albicans	16	17	18	18	17.25
Mean ethyl acetate Lepidium sativum seed extract	12.8	13	15	16.2	

All different *Lepidium sativum* seed extract (aqueous, petroleum ether, chloroform and ethyl acetate) have the high activity against *Candida albicans* fungus these result agree with those who obtained that seed extract of garden cress have potent effect against bacteria and fungi [21,22].

Table 6. Inhibition zone (in mm) for different concentrations of Lepidium sativum petroleum ether seed extract

Microorganism	Concentration of the Lepidium sativum seed extract by ethyl acetate				Mean microorganism
	12.5	25	50	100	
Escherichia coli	14	14	15	15	14.5
Pseudomonas aeruginosa	12	12	13	13	12.5
Salmonella typhimurium	13	13	14	14	13.5
Bacillus cereus	-	-	-	-	-
Candida albicans	13	13	14	14	13.5
Mean petroleum ether Lepidium sativum seed extract	10.4	10.4	11.2	11.2	

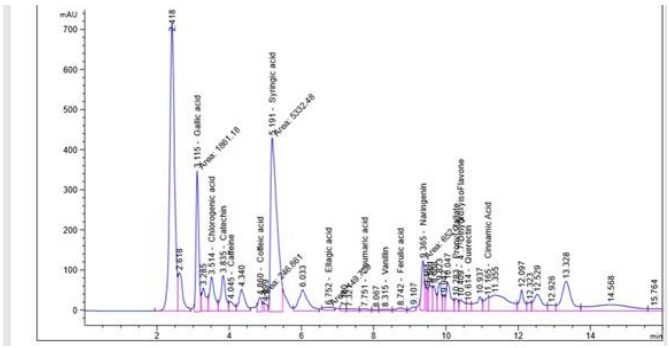


Figure 1. Polyphenol constituents of *Lepidium sativum* aqueous seed extract identified by HPLC

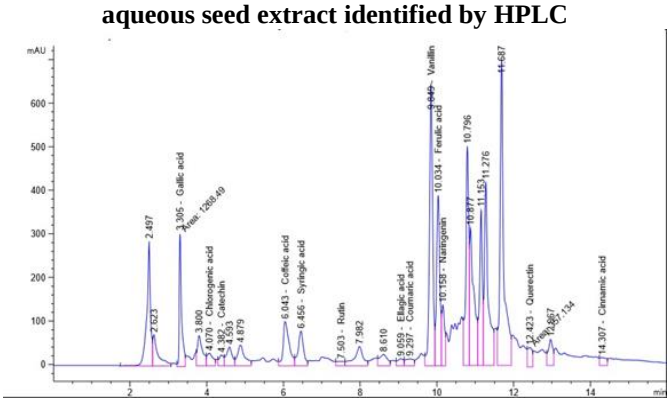


Figure 2. Polyphenol constituents of *Lepidium sativum* ethyl acetate seed extract identified by HPLC

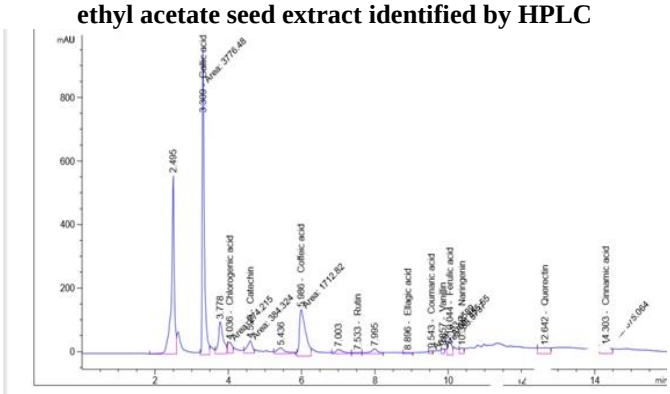
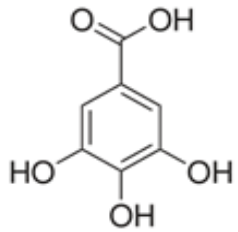
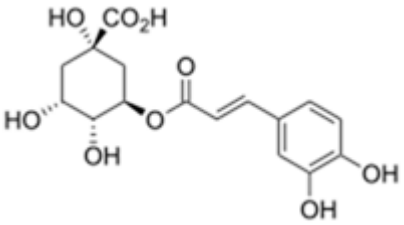


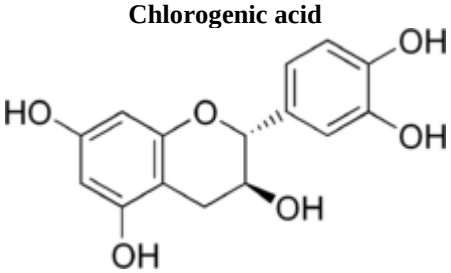
Figure 3. Polyphenol constituents of *Lepidium sativum* petroleum ether seed extract identified by HPLC



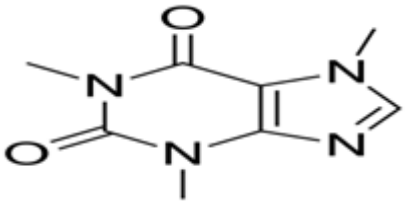
Gallic acid



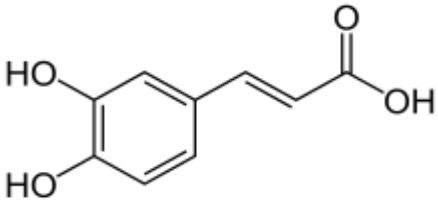
Chlorogenic acid



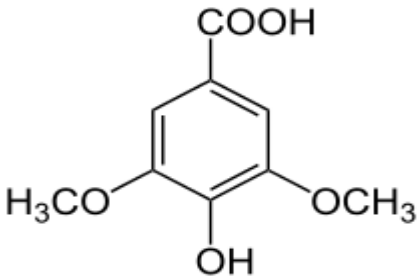
Catechin



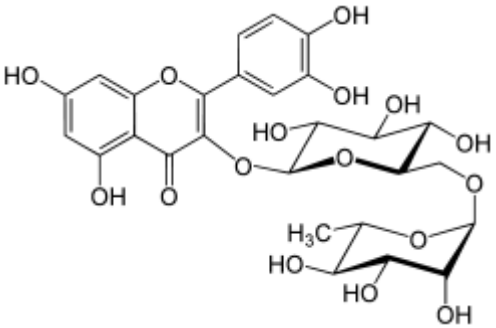
Coffeine



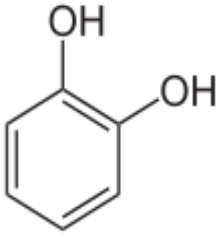
Coffeic acid



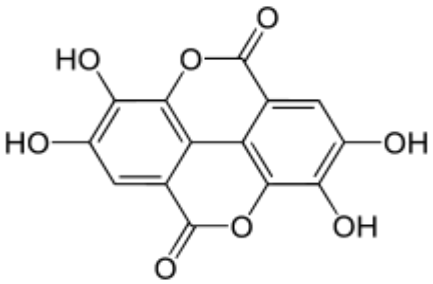
Syringic acid



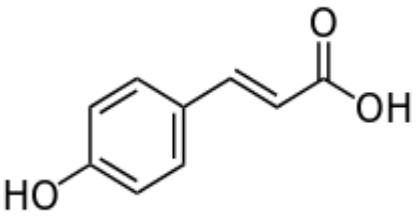
Rutin



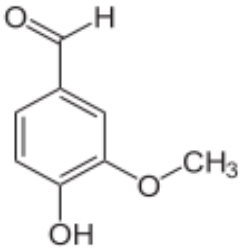
Pyro catechol



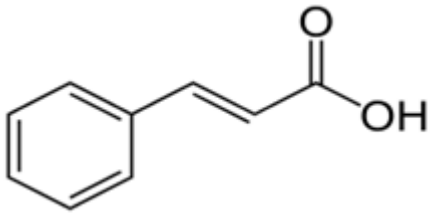
Ellagic acid



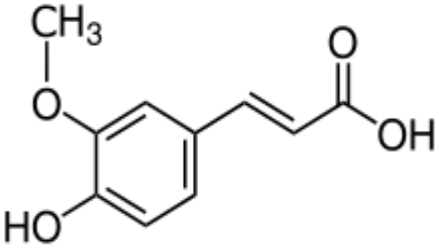
Coumaric acid



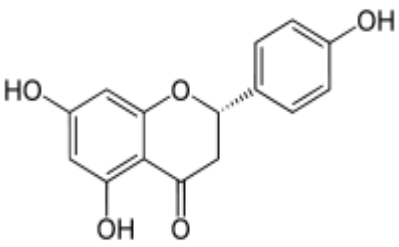
Vanillin



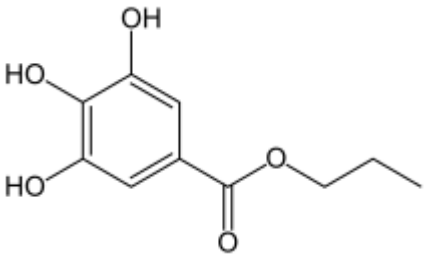
Cinnamic Acid



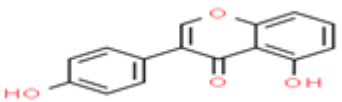
Ferulic acid



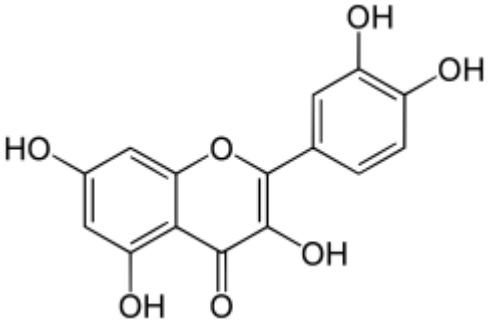
Naringenin



Propyl Gallate



4`.7-DihydroxyisoFlavone



Querectin

Figure 4. Polyphenols Chemical Structures

Conclusion

The *Lepidium sativum* contains a polyphenol compound had a potent antioxidant, antibacterial and antifungal activity in all different leaf extract.

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Data Availability

All datasets generated or analyzed during this study are included in the manuscript and/or the Supplementary Files.

Ethics Statement

This article does not contain any studies with human participants or animals performed by any of the authors.

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