

Qualitative and Quantitative Determination of Secondary metabolites and Antioxidant Potential of *Eruca sativa*

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Abstract

Objective: To Determine the phytochemical composition of *E. sativa* (stem, leaves, flowers and seeds), and evaluate their antioxidant activity.

Method: Preliminary phytochemical screening for all parts of *E. sativa* (stem, leaves, flowers and seeds) was carried out according to standard methods. Total phenolic contents of all methanolic extracts of *E. sativa*, have been quantified spectrophotometrically. Hydrogen Peroxidase and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical assays have been used to analyze antioxidant characteristics of all extracts of *E. sativa* (leaves, stem, seed, flowers and seeds). Further separation and identification of number of phenolic compounds has been carried out by Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC).

Results: Experimental evaluation indicated that *E. sativa* is a rich source of secondary phytoconstituents (Alkaloids, flavonoids, Diterpenes, Coumarins, polyphenols, tannins, cardiac glycosides etc). Quantification of total phenolic contents from all aerial parts revealed that they contain significant amount of phenolics particularly seeds and leaves (27.1 ± 0.2 mg, 23.07 ± 0.11 GAE/g) respectively. Separation and identification of phenolics from *E. sativa* stem, leaves, flowers and seeds extracts through RP-HPLC showed presence of variety of important phenolics namely; Vanillin (RT=3.853), Ellagic acid (RT=4.04), Salicylic acid (RT=19.09), Resorcinol (RT=3.30), Catechol (RT=3.53), Quercetin (RT=18.91), Benzoic acid (RT=10.4), Tannic acid (RT=5.06), Kaempferol (RT=8.70) and Rutin (RT=9.2).

Conclusion: Results revealed that *E. sativa* is a rich source of secondary phytoconstituents which impart significant antioxidant potential. This work also contributes significantly to support the claim about the use of this herb in folk medicines. Further investigation regarding isolation and purification of a number of phytoconstituents from leaves, stem, flowers and seeds of *E. sativa* may yield optimal combinations of therapeutic alternates.

Keywords: Phytoconstituents; Antioxidant potential; *E. sativa*; RP-HPLC; Phenolics

Introduction

Eruca sativa known as *Taramira* (family Brassicaceae), is a minor oil crop and medicinal plant in various parts of Middle East and Indo-Pak subcontinent. Since long it has been used in traditional medicines as remedies for different diseases [1]. Therefore phytochemical composition and respective biological activities are important to understand the therapeutic potential of medicinal herbs. Among other, phenolic compounds are the most widely explored phytochemicals for therapeutic potential in different medicinal plants. Most of these studies conclude that pharmacological activities of any medicinal plant are due to the presence of secondary metabolites. Secondary metabolites usually consist of the phenolic compounds, alkaloids, tannins, saponins, carbohydrates, glycosides, flavonoids, steroids, etc. Most phenolic compounds such as flavonoids, glycosides, triterpenoids, flavonols, carbohydrates and anthraquinones are commonly present in most of the medicinal plants. All of these secondary metabolites and particularly phenolic compounds have been reported as scavengers of free radicals and also have been considered as good therapeutic candidates for free radical related pathologies [2]. Nowadays, there is an increasing focus for the search of anti-oxidants (non-synthetic) from medicinal plant such as carotenoids, ascorbic acid (vitamins), phenolic and flavonoids. There are also several studies related to the analysis of phenolic composition in other members of the Brassicaceae family [3-6]. So far, whole plant extracts and purified Glucosinolates (GSLs) *Brassica oleracea* L. has been studied for their antioxidant potential by evaluating its effects on lipid peroxidation (deoxyribose assay), the Bleomycin assay and a Radical cation 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS) [7]. It has been reported that *E. sativa* seed extracts are potent antioxidants, exhibit diuretic effects and provide renal protection. Previous phytochemical studies of *E.*

sativa showed that leaves and seeds contain glucosinolates. Three new quercetins have been isolated and identified from *E. sativa* leaves [8]. However, there is not sufficient information in the form of scientific analysis about detailed phytochemical composition of *E. sativa* and their respective bioactivities [1]. Anti-oxidative activity is a measure of capability of compound to scavenge free Hydroxyl groups and Oxygen species. It is very important property of medicinal plants because there are number of reports which mention that in biological systems, free radicals are causative agents for different disease such as cancer. Therefore, antioxidant properties are an index of antioxidant potential against reactive oxygen species (free radicals) [2].

Our early studies showed that *E. sativa* seeds are rich source of phytochemicals including flavonoids, phenolics, alkaloids and ascorbic acid [9]. Traditionally, in folk medicine people also use leaves, stem and inflorescence of *E. sativa* in their food [2]. So this prompted us to investigate all part of plant to identify its nutritional value. Therefore this study was conducted with the aim to investigate phytoconstituents present in all aerial parts of *E. sativa* (stem, leaves, flowers and seeds) and assessment of their antioxidant characteristics. Further, considering

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the reported presence of phenolics in of *E. sativa*, we decided to carry out the isolation and identification of different phenolic compounds through Reversed-phase high-performance liquid chromatography (RP-HPLC). RP-HPLC exhibits a great potential in separating complex mixtures of phenolic compounds [10,11] by using combination of mobile phases with different elution gradients and specific run times.

Material and Methods

Collection and identification of plant sample

Crude oil, Seeds and Fresh plant sample of *E. sativa* consist of all aerial parts were purchased from the local herbal store (district Islamabad, Punjab) on January, 2013 and specimen was identified by Dr. Muhammad Qasim Hayat in the Department of Plant Biotechnology, Atta-ul-Rehman School of Applied Biosciences, National University of Science and Technology Islamabad, Pakistan. The identified voucher specimen (No. 23) was deposited in the Medicinal Plant Research Laboratory for future record. All plant samples were dried under shade and then ground into fine powder form (80 mesh sieve size) by electrical grinder. Powdered sample of all parts stored in clean paper bags [12] and preserved at 4°C for further analysis.

Preparation of plant extracts

Organic extracts of seeds and aerial parts of plant (leaves, stem and flowers separately), were prepared by using two different solvents (85% Methanol and water with increasing polarity) [13]. Dried plant powder weighed carefully and used for extract preparation through soxhlet apparatus at respective temperature. The extract obtained was filtered and concentrated in rotary evaporator [14,15]. The concentrated plant extracts (semi-solid mass) were lyophilized and than store the dried organic extracts in air tight brown bottles.

Qualitative analysis of secondary phytochemicals

Extracts of all plant parts (seeds, stem, leaves and flowers separately) and crude oil were evaluated for preliminary screening of secondary phytochemicals such as, alkaloids, polyphenols, phytosterol [16], flavonoids, Diterpenes, cardiac glycosides, Coumarins, Leucoanthocyanins, Anthocyanin, Tannins, Steroids, Terpenoids [17-20] and saponin [21] following the reported methods with minor modifications.

Estimation of Total Phenolics content in different parts of *E. sativa*

Folin-ciocalteu method has been used for analysis of total phenol from all plant samples (leaves, stem, flowers and seeds) after extraction. Organic extracts of plant powder (1 gm) were prepared in combination of solvents (20 mL) acetone-methanol-water with the ratio of 7:7:6 volume/volume. Afterward these extracts were subjected to centrifugation (6000 rpm, 10 mins). Than analyzed for quantification of total phenolic contents following the methodology [22,23]. 1 mL of organic extract, 10 mL of water (deionized) and 2 mL of Folin-Denis reagent has been taken in test tube. Saturated sodium carbonate solution (2 mL) added to reaction mixture and kept in dark at room temperature for 1 hour of incubation. Absorbance was measured at spectrophotometrically at 640 nm. The total phenolics concentration was calculated from a calibrated curve of standard phenolic compound Gallic acid and phenolic contents of plant extracts were expressed as mg GAE/g, Gallic acid equivalent.

Antioxidant activity

DPPH radical scavenging activity: DPPH (1,1-diphenyl-2-

picrylhydrazyl) scavenging activity of all extracts (leaf, stem, flowers and seeds of *E. sativa*) were measured by the spectrophotometric method for the presence of DPPH as a free radical. Plant extract (25 µl) is added into 975 µL DPPH solution, which is prepared by dissolving 2.5 mg DPPH in 100 mL of Methanol. Whole mixture was shaken vigorously and then subjected for incubation (30 min) in the dark place at the room temperature. Finally measure the absorbance at 517 nm through spectrophotometer. Following equation has been used to calculate the percentage of DPPH scavenging activity:

$$\text{Percentage DPPH scavenging activity} = \frac{(\text{A control} - \text{A Sample}) \times 100}{\text{A control}}$$

Whereas

A control = Absorbance of the control sample

A sample = Absorbance of the sample containing plant extract/standard.

Antioxidant activity of all different extracts of *E. sativa* have been expressed in term of IC50 values, which is specific concentration of the extract that caused 50% neutralization of DPPH radicals [24]. Calibration curve for Ascorbic acid (vitamin C) used as a standard [25].

Hydrogen peroxide scavenging activity: H₂O₂ Scavenging potential of plant extracts was analyzed by reported method [26,27] with slight modifications. H₂O₂ solution has been prepared in PBS (Phosphate Buffer Saline). 0.6 mL of 4 mM H₂O₂ has been added to 4mL of extract and incubated for 10 min. The absorbance of whole mixture was measured at 230 nm through spectrophotometer. Following formula has been used to estimate % age of H₂O₂ free radical scavenging activity. Ascorbic acid has been taken as standard.

$$\text{Percentage H}_2\text{O}_2 \text{ scavenging activity} = \frac{(\text{A control} - \text{A Sample}) \times 100}{\text{A control}}$$

Whereas

A control = Absorbance of the control sample

A sample = Absorbance of the sample containing plant extract/standard.

Reversed-phase high-performance liquid chromatographic identification of Phenolics from *E. sativa*

Sample preparation for RP-HPLC analysis: Powdered plant samples (200 mg) was subjected for extraction with 6 mL of hydrochloric acid (25%) and 20 mL methanol for 1 h. Plant extracts were filtered into volumetric flask. Then combine the whole extract, filter and dilute with HPLC grade methanol. Take 5 mL of extract and filter through a Chromafil, transfer extract into a volumetric flask and further diluted with HPLC grade methanol (up to 10 mL) [28].

Specification of the RP-HPLC instrument: Analysis of all samples was performed using RP-HPLC, Perkin Elmer Series 200 pump and equipped with 200 UV/VIS detectors, Total chrom V2.6.0.01 with LC instrument control software having reverse phase water guard Column: Symmetry C18 (5µm, 250mm) and Hamilton microliter syringe using an injection volume of 20 µl. RP-HPLC methods is consist of gradient elution of two solvents - Solvent A (Methanol) and Solvent B (Acetic acid in water (1:25)). Detector wavelengths range set at 280 nm and 360 nm. Flow rate adjusted at 1.0 ml/min. The gradient program start with 100% B and then held at this concentration for the first 4 minutes. This was followed by 50% eluent A for the next 6 minutes after which

concentration of A was increased to 80% for the next 10 minutes and then reduced to 50% again for the following 2 minutes. Therefore total run time was of 22 minutes. Standards: The standard phenolic compounds including Quercetin Dihydrate, Salicylic Acid, Aspirin, L-Ascorbic Acid, Tannic Acid, Benzoic Acid, Gallic Acid, Catechol, Vanillin, Ellagic Acid, Phloroglucinol GR, Rutin, Kaempferol and Resorcinol were purchased from reputed manufacturers. Preparation of standard samples: Standard compound (6 mg) was dissolved in HPLC grade Methanol (10 mL). This mixture was subjected to sonication for mixing. Before injecting into the column this mixture was filtered by using Whatman Nylon Membrane Filter (0.45 µm). The HPLC analysis of 14 standard phenolic compounds was performed at the same wavelength, flow rate and sample concentration [2,28,29].

Statistical analysis: Statistical evaluation of results was carried out by applying student's *t*-test, and results were analyzed as mean ± SD (Standard Deviation) and percentage values of different Phytochemicals. Statistical Significance of results was considered at P-values, 0.05 and 0.01. Concentrations yielding 50% inhibition (IC50) was calculated by interpolation from linear regression analysis. All statistical analyses have been performed by using Graph Pad Prism 5 software.

Results and Discussion

Vegetables belonging to family *Brassica* are rich in phytochemical constituents particularly polyphenols and they are known to play an important role in human nutrition. We analyzed three different extracts (methanol, water and methanol-water) from leaves, stem, flowers and seeds of *E. sativa* for evaluation of secondary metabolites. We have measured the antioxidant activity of these extracts and correlated the data with total phenolic compounds. Further, we have done identification for different phenolics compounds present in extracts of *E. sativa* (stem, leaves, seeds and flowers) through RP-HPLC. Qualitative analysis of secondary phytoconstituents has been carried out from aqueous and methanolic extracts of stem, leaves, flower, seeds and crude oil. These results showed that all parts of *E. sativa* are

rich source of Steroids, Terpenoids, Tannins, Diterpenes, and Cardiac glycosides, Polyphenols, Alkaloids, Flavonoids and Phytosterols (Table 1). Saponins are only present in extracts of seed and leaf. While Leucoanthocyanins and Anthocyanin are absent from all aerial parts. Comparative phytochemical analysis of aerial parts of plants with crude oil which is available at local herb stores, indicate that crude oil contain less number of phytoconstituents, might be due to the method of extraction. Crude oil contains only steroids, terpenoids, coumarins and flavonoids. Previous study related to phytochemical analysis of *E. sativa* seeds report similar findings [30]. Therefore presence of significant amount of phytoconstituents confers medicinal properties, including antioxidant activities on these extracts of *E. sativa*.

Qualitative analysis showed that all aerial parts of *E. sativa* are rich in Phenolics, which are medicinally important phytoconstituent [2]. Therefore Folin-ciocalteu method has been used to estimate total Phenolic from all different parts of *E. sativa*. Results showed that seeds are rich in phenolics as they contain highest amount of phenolics 27.1 ± 0.2 mg GAE/g extract as compared to all other aerial parts. *Eruca* leaf and flower also contain significant amount of phenolics 23.07 ± 0.11, 19.9 ± 0.3 mg GAE/g respectively. Results indicate that whole plant is a good source of phenolics which support its use in most of the regions where people consume this herb as a whole plant (leaf, flowers, stem and seed) or various combinations in the form of fresh salad. Antioxidant potential of different extracts of *E. sativa* (leaves, seeds, stem and flowers) has been evaluated by DPPH radical scavenging assay and compared with Gallic acid as a standard phenolic compound. Seeds extract has highest antioxidant potential at 50 mg/mL concentration (66.93 ± 0.2). Antioxidant activity of the tested extracts and the positive control (vitamin C), expressed as the percentage of deactivation of the DPPH free radicals (Table 2). Comparatively radical scavenging potential in all extracts follow this trend seeds>leaves>flowers>stem (Table 3). Antioxidants potential of phenolics in the extracts was determined by the IC50 values (the concentration of the sample required to scavenge 50% of the DPPH free radicals). High antioxidant activity and phenolic content observed in seeds extracts of *E. sativa*. Methanolic seeds extracts also exhibit lowest IC50 (100.6 ± 0.21) among all

S. No	Plant Sample (<i>Eruca sativa</i>)		Phytochemicals												
			Steroids	Terp enoids	Tannins	Diterpenes	Saponins	Cardiac glycoside	Coumarins	Leucoan-thocyanins	Anthocyanin	Polyphenols	Alkaloids	Flavonoids	Phytosterol
1	Leaf	80% Meth	+++	+++	+++	+++	ND	+++	+++	ND	ND	+++	++	+++	+++
		Aq	+++	+++	++	+++	+++	++	+++	ND	ND	++	+++	++	++
2	Stem	80% Meth	+++	+	++	++	ND	+	+	ND	ND	++	+	+	+
		Aq	+	ND	+	++	+	ND	+	ND	ND	+	+	+	+
3	Flower	80% Meth	+++	+++	++	+++	ND	+++	+++	ND	ND	+++	+	+++	+++
		Aq	+++	+++	+	+++	ND	+++	++	ND	ND	+	+	+	++
4	Seed	80% Meth	+++	+++	+++	++	ND	+++	++	ND	ND	+++	++	++	+++
		Aq	+	+	ND	+++	+++	++	ND	ND	+	+++	+	+	+
5	Whole Plant Extract	80% Meth	+++	+++	+++	+++	++	++	+++	+	ND	+++	++	+++	+++
		Aq	+++	+++	+	++	ND	ND	+	ND	ND	+	++	+	++
6	Crude Oil		+++	+++	ND	+	ND	+++	+++	ND	ND	ND	ND	+++	ND

+ - slight presence; +- medium presence; +++- heavy presence; ND- Not detected; 80% Meth, 80% Methanolic extract. Aq

Table 1: Qualitative analysis of secondary Phyto-chemicals in different parts of *Eruca sativa*.

S.No	Plant Extracts (<i>E. sativa</i>)	Concentration (µg/ml)	DPPH Radical Scavenging activity in (% age)	Free Radical H ₂ O ₂ Scavenging activity in (% age)
1.	Stem extract	25	2.25 ± 0.1	1.3 ± 3.4
		50	4.85 ± 0.2*	2.8 ± 0.5
		75	15.76 ± 0.01	5.6 ± 0.9
		100	27.30 ± 0.2	23.0 ± 0.7
		125	23.65 ± 0.11*	43.5 ± 1.9
		150	24.63 ± 1.17	44.0 ± 5.6
		175	34.40 ± 2.3	41.1 ± 0.6
2.	Leaf extract	25	5.15 ± 0.71*	3.7 ± 1.1
		50	4.79 ± 0.66	6.7 ± 0.2
		75	8.85 ± 0.45	7.12 ± 0.8*
		100	14.5 ± 0.3*	11.0 ± 2.3
		125	33.06 ± 0.72	22.13 ± 5.5
		150	40.84 ± 1.52	24.44 ± 1.4
		175	44.95 ± 0.24*	31.11 ± 0.7
3.	Flower extract	25	2.53 ± 0.87	4.22 ± 1.9
		50	2.77 ± 0.10	4.21 ± 2.0
		75	15.46 ± 1.17	11.41 ± 2.6
		100	20.20 ± 1.3	30.0 ± 0.6
		125	28.53 ± 1.00	37.43 ± 0.5
		150	41.078 ± 0.91*	44.9 ± 0.5*
		175	49.44 ± 3.05	55.4 ± 1.3
4.	Seeds extract	25	5.58 ± 0.4	7.00 ± 1.2
		50	16.93 ± 0.3	17.00 ± 1.7
		75	28.01 ± 1.01	18.23 ± 5.1
		100	37.69 ± 2.89	24.55 ± 4.1*
		125	37.69 ± 0.66*	41.09 ± 3.4
		150	44.80 ± 0.78*	47.07 ± 0.9
		175	52.91 ± 1.89*	51.11 ± 0.8
5.	Ascorbic acid	25	9.70 ± 1.59*	3.15 ± 1.31
		50	19.38 ± 1.85*	9.44 ± 2.0
		75	23.45 ± 1.03*	10.21 ± 2.8*
		100	33.45 ± 0.68*	11.78 ± 1.52
		125	44.59 ± 1.85	26.00 ± 0.27
		150	57.85 ± 0.64	39.10 ± 0.56
		175	74.59 ± 1.20*	56.09 ± 1.65
		200	84.59 ± 0.62*	77.17 ± 2.22*

Significance of results evaluated at * P<0.05, **P<0.01. Values are in term of Mean ± SD after triplicate analysis (n=3)

Table 2: Percentage (%) DPPH radical scavenging activity of methanolic extracts (stem, leaves, flower and seeds) of *E. sativa* and Ascorbic acid.

other extracts. Recent studies [31] suggested that the ethanolic extracts of *E. sativa* seeds possessed a potent antioxidant activity and exert a protective effect on mercuric chloride induced renal toxicity. Studies report that these health-promoting activities of *E. sativa* are partially related to their strong antioxidant properties [32-34].

Free radical H₂O₂ scavenging potential of all plant extracts including stem, leaves, seeds and flowers has been evaluated spectrophotometrically (Table 3). Results indicate that seeds, and leaf extracts have highest scavenging potential at the concentration of 200 µg/mL (64.12 ± 4.6, 56.2 ± 1.1 respectively). Although Hydrogen peroxide (H₂O₂) is a weak reactive agent, but in biological systems it

is also a source of free hydroxyl radicals, so production of more HO- (Hydroxyl groups) free radicals disturb the normal balanced HO- level in biological systems, hence it cause toxicity in the cells. Therefore, the eliminating excessive amount of H₂O₂ is very important for antioxidant defense in cell [26]. Recent scientific reports revealed the role of free radicals in the emergence of such lifestyle diseases such as atherosclerosis, heart attack, stroke, cancer, diabetes, senile cataracts and accelerated aging. Structurally polyphenols contain number of Hydroxyl groups (HO-), which are responsible for antioxidant characteristics and potential of polyphenols. Because of these OH-groups, polyphenols are able to chelate transition metal ions, such as iron, and copper. Generally, these metal ions are involves to initiate various free radical chain reactions. Additionally they are also capable to inhibit enzymes such as xanthine oxidase and NADPH oxidase. These enzymes catalyze the production of large amounts of reactive oxygen [23]. In present study we are reporting the phenolic composition in methanolic extracts of different parts of *E. sativa* (stem, leaves, flowers and seeds) for the first time. Four different extracts of *E. sativa* has been analyzed and eleven different phenolics have been identified through RP-HPLC. There is limited data on the phenolic and flavonoid content of *Eruca* species. Qualitative analyses by liquid chromatography/mass spectrometry (LC/MS) and NMR of identified quercetin triglycosides in *E. sativa*. Initial qualitative thin-layer chromatography analysis identified various isorhamnetin glycosides, kaempferol and quercetin in leaves of flowering *Diplotaxis* species [33,35]. Limited literature is present related to the chemical constituents of *E. sativa* leaves and flowers [35], which do not provide the sufficient information. In present study RP- HPLC conditions, provide good separation of the peaks which could be identified in the chromatogram and revealed that different types of phenolic compounds are present in different parts of *E. sativa*. These phenolic compounds were identified in comparison

S. No	Plant Extracts (<i>E. sativa</i>)	IC50 (µg/mL)	Total content of Phenolics compounds (mg GAE/g)
1.	Stem extract	212.8 ± 1.23*	13.55 ± 1.1
2.	Leaf extract	101.0 ± 0.4	23.07 ± 0.11
3.	Flower extract	101.0 ± 0.11	19.9 ± 0.3
4.	Seeds extract	100.6 ± 0.21*	27.1 ± 0.2
5	Ascorbic acid	33.67 ± 1.20	-

Table 3: IC50 values and total content of phenolic compounds in various methanolic extracts (stem, leaves, flower and seeds) of *E. sativa* and Ascorbic acid.

S.No	Peak Name	RT	Area	% Area	Height
1.	Vanillin	3.853	4305894.50	54.17	81589.66
2.	Ellagic acid	4.040	3082201.98	38.78	79083.68
3.	Salicylic acid	19.02	494722.70	6.22	15958.69

RT=Retention time in minutes

Table 4: HPLC profile of phenolics peaks of *E. sativa* stem extract.

S.No	Peak Name	RT	Area	% Area	Height
1.	Resorcinol	3.30	17354.29	1.73	2169.17
2.	Catechol	3.53	17632.81	1.75	2948.24
3.	Vanillin	3.805	7189.74	0.71	3216.00
4.	Ellagic acid	4.174	158858.88	15.80	7772.43
5.	Rutin	9.086	12179.55	1.21	2570.01
6.	Benzoic acid	10.4	123255.34	12.26	6640.09
7.	Quercetin	18.91	93610.73	9.31	6190.89
8.	Salicylic acid	19.70	4829.08	4.8	542.17

RT= Retention time in minutes

Table 5: HPLC profile of phenolics peaks of *E. sativa* flower extract.

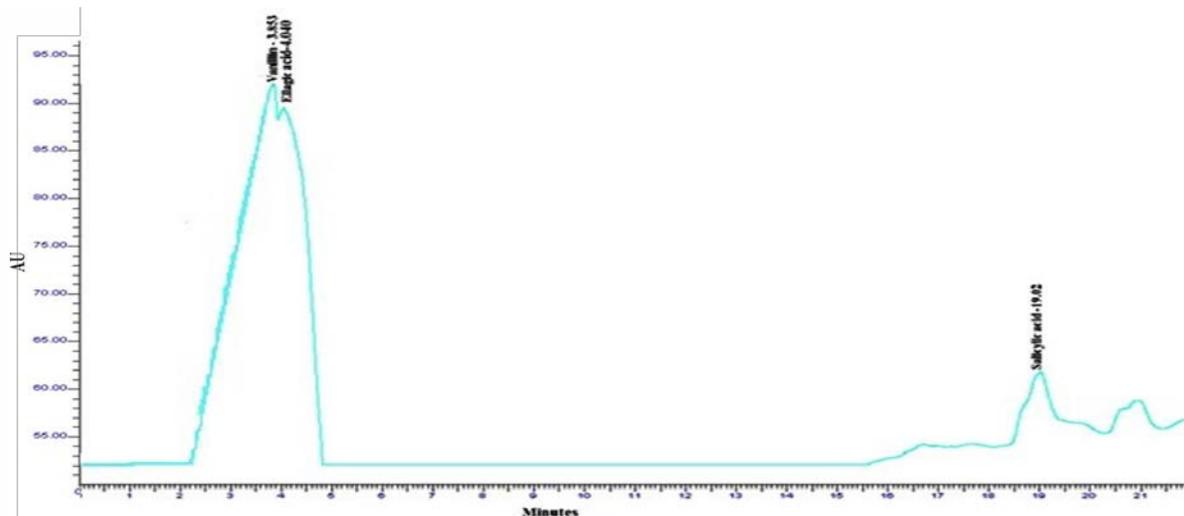


Figure 1: HPLC profile of phenolic peaks of *E. sativa* stem extract.

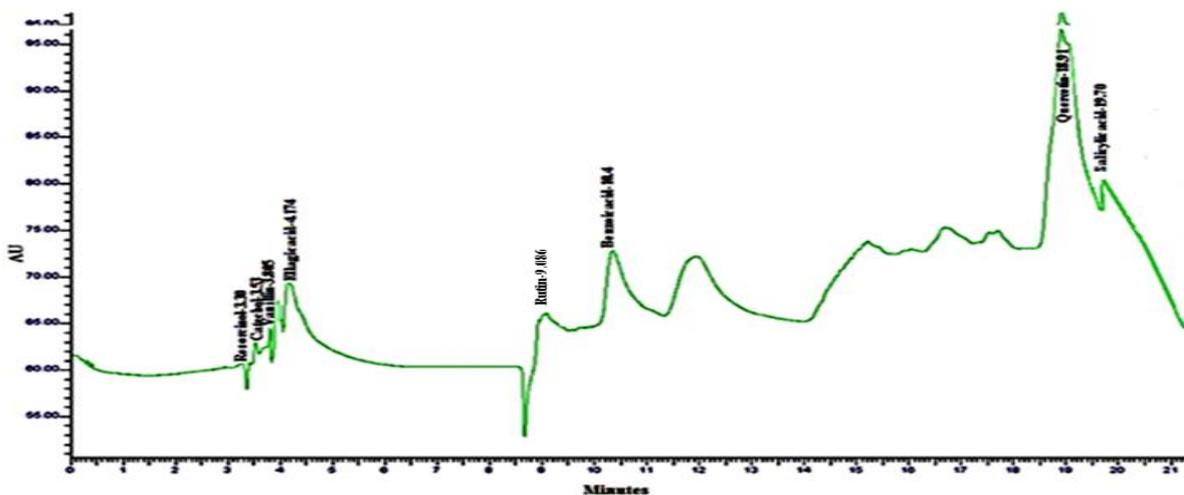


Figure 2: HPLC profile of phenolic peaks of *E. sativa* flower extract

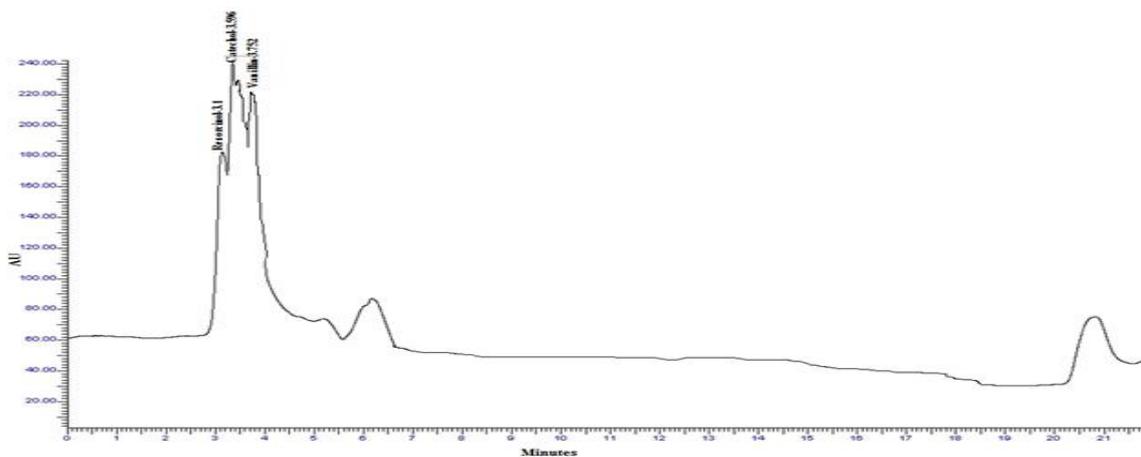


Figure 3: HPLC profile of phenolic peaks of *E. sativa* leaf extract.

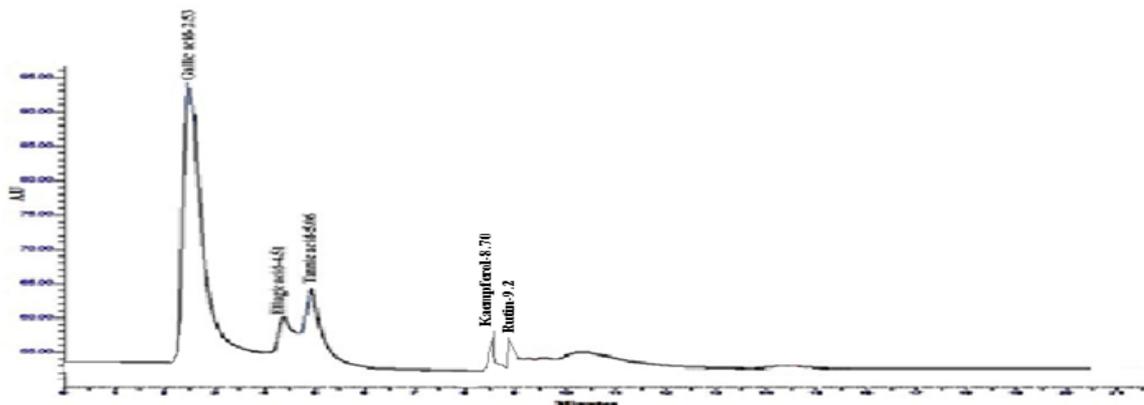


Figure 4: HPLC profile of phenolic peaks of *E. sativa* seed extract

S.No	Peak Name	RT	Area	% Area	Height
i	Resorcinol	3.1	1548119.19	12.46	116961.81
ii	Catechol	3.596	827127.81	6.66	150518.70
iii	Vanillin	3.752	3436548.29	27.67	151573.40

RT= Retention time in minutes

Table 6: HPLC profile of phenolics peaks of *E. sativa* leaf extract.

S.No	Peak Name	RT	Area	% Area	Height
i	Gallic acid	2.53	93193617.27	54.87	1931.77
ii	Ellagic acid	4.51	13840349.10	8.15	566828.51
iii	Tannic acid	5.06	25619886.11	15.08	901591.19
iv	Kaempferol	8.70	567711.00	12.2	345556.0
v	Rutin	9.2	125672.33	6.7	45666.12

RT= Retention time in minutes

Table 7: HPLC profile of phenolics peaks of *E. sativa* seed extract.

with reference compounds under same conditions and UV spectrum. Initially separated phenolics peaks were identified by direct comparison of their retention times with those of respective standards compounds.

Later on standard solution was added into the sample and peaks were identified by the observed increase in their intensity and this procedure was performed for each standard compound separately. Chromatogram for *Eruca* stem extract (Figure 1; Table 4) showed peaks of vanillin (RT=3.853), ellagic acid (RT=4.04) and salicylic acid (RT=19.09). Phenolic profile of flowers consists of (Figure 2; Table 5) vanillin (RT=3.805), Resorcinol (RT=3.30), Catechol (RT=3.53), ellagic acid (RT=4.174), salicylic acid (RT=19.70), rutin (RT=9.086), Quercetin (RT=18.91) and Benzoic acid (RT=10.4). Only three phenolic compounds has been identified in chromatogram of leaves (Figure 3; Table 6) vanillin (RT=3.752) Resorcinol (RT=3.1) and Catechol (RT=3.596), while two other peaks were remained unidentified. Chromatogram of *Eruca* seed extract showed peaks (Figure 4; Table 7) for gallic acid (RT=2.53), ellagic acid (RT=4.51), tannic acid (RT=5.06), kaempferol (RT=8.70) and rutin (RT=9.2).

Conclusion

Quantitative analysis of phenolics from *E. sativa* was achieved first time in this work. The observed level of phytoconstituents revealed that *E. sativa* is a rich source of antioxidant compounds. The RP-HPLC

analysis of phenolics enables reproducible and accurate determination of eleven common phenolic compounds from *E. sativa*.

Conflicts of Interest

All authors have no conflict of interest.

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