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Research Article

Physico- chemical investigation and antioxidant activity studies on extracts of *Eruca sativa* seed

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Abstract

Along with the other medicinal plants Eruca sativa has remarkable potential against various diseases. Present work is based on the estimation of antioxidant potential of dist. Water, ethanolic and methanolic extracts of *E. sativa* seed oil. On phytochemical determination it was exposed that different plant extracts/fractions contains alkaloids, flavonoids, saponins, tannins, phenols, carbohydrates, steroids and proteins. Its physico-chemical screening was concluded the saponification value and fat value of the *E. sativa* seed oil. Antioxidant activity was evaluated by using DPPH free radical scavenging scheme. The maximum (30.60±1.1) anti-oxidative potential was exhibited by aqueous extract of *E. sativa* seed oil. IC50 was calculated maximum 126.2 in eq. extract of *E. sativa* seed oil. From current study, it is concluded that this plant seed oil could be used as a source of natural antioxidant and medicinal purposes.

Keywords: Medicinal plants, Eruca sativa, Antioxidant, Alkaloids, Quercetin, DPPH free radical assay

1. Introduction

In recent years, because of increasing local, national and international interest, the demand for medicinal and aromatic plants increases many folds in both developing and developed countries[1]. According to World Health Organization (WHO), more than 80% of the world's population still relies on traditional medicines for their basic health needs. Medicinal plants contribution in disease prevention and control has been attributed to antioxidant properties of their constituents, broadly termed as polyphenolic compounds. In addition to their role as antioxidants, these compounds possesses broad spectrum of medicinal properties, such as anti-inflammatory, ant-microbial, anti-thrombic, cardio-protective and vasodilatory[2].

Scientists are taking keen interest in correlating phytochemical constituents with their pharmacological activities. Phytochemicals such as flavonoids, terpenoids, lignans, glycosides and various alkaloids are well distributed in plant kingdom and analyzed scientifically for its antiallergic activity[3-4].

Eruca sativa locally known as Taramira is grown in different parts of Indo-Pak subcontinent. It has been cultivated since ancient times in southern Europe and Central Asia. *Eruca sativa* Mill commonly referred to as "rocket salad, argula, roquette, or white pepper in English is a member of brassicaceae family. It is minor oil crop and used in traditional medicines as remedies for different diseases. Its oil is mainly used in industry in soap making, as an illuminating and lubricating agent, in massage and in medicines as well[5-6]. The extracted oil from the seed of *E. sativa* is prohibited for eating purposes because of its pungent and obnoxious odour. The cake is used as manure for improving the soil physical condition fertility and can also be used as nutritional feed for animals[7].

E. sativa seed extract contains important secondary metabolite such as flavonoids, alkaloids, tannins, phenols, saponins, ascorbic acid and those are used as remedies of many diseases and frequently required in traditional medicines. Essential oil especially erucic acids was present in high concentration those are responsible for antibacterial activity, which could be used for the preparation of drugs required for human and animal health[8].

In traditional medicines, rocket species are recognized for their therapeutic properties such as astringent, digestive, diuretics, tonic, laxatives, rubefacient and stimulants etc. Rocket salad species are rich in antioxidant compounds, as a source of vitamins like ascorbic acid, carotenoids as well as polyphenols[9].

Missiry and Gindy (2000) proposed that *Eruca sativa* seeds exhibits antidiabetic effect by reducing oxidative stress experimented in rats. In addition ethanolic extract of *Eruca sativa* possesses significant anti-secretary, cytoprotective, and antiulcer activities against gastric lesions experimentally induced in rats by elevating mucus synthesis and endogenous prostaglandins through its effective antioxidant activity[10].

Present study focus on the phytochemistry of ethanlic and methanolic extract of *E. sativa* seeds along with their antioxidant power.

2. Experimental

2.1. Collection and preparation of seed samples

Eruca sativa seed samples were purchased from local herbal store of Rawalpindi. Seeds samples were dried in shade and followed by drying and finally converted into powered form (40 mash). This powder was again dried in the pre-set incubator at 50 ^{o}C for the removal of its

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moisture content. The samples was preserved in the sterilized clear bag, labeled properly and stored carefully in the refrigerator at 4°C for further processing.

2.2 Proximate analysis

The seed samples of *E. sativa* were analyzed for protein, moisture, fiber, carbohydrates and ash contents by using AOAC, 1990.

2.2.5 Fat Content

The powered plant sample was analyzed for fat content according to standard method of AOAC (1990) and the results were expressed in percentage[11].

2.2.6 Estimation of Carbohydrate

The method used in this study is the modification of the protocol suggested by Khalifa[12].

2.3 Preparation of plant extracts

2.3.1 Hot Water Extraction

About 5 gm of finely powdered seed sample was placed in a beaker and added in it 200 mL of D.H_2O. Mixture was well shaken and heated at about 30º- 40ºC for about 20 minutes with constant stirring. Aqueous extract was filtered and was additionally used for phytochemical screening. The water extract was stored in air tight container and kept in refrigerator for further studies.

2.3.2 Solvent Extraction

About 25 gm of powdered plant material was evenly packed into a thimble and subjected to Soxhlet extraction method by using 250 mL of organic solvents (methanol and ethanol) separately. Extraction was done under normal conditions at room temperature. The extraction process continues since the solvents develop into colorless. Extract recovered was filtered and heated at 30-40ºC on hot plate till evaporation. Dried extract was stored at 4ºC for future use and analysis of phytochemical.

Extracts prepared were analyzed for the screening of bioactive compounds by standard methods[13-15].

2.4 Qualitative estimations of phytochemicals

The identification or presence of Protein, Alkaloids, Reducing Sugars, Flavonoids, Phenols and Tannins, Steroids, Glycosides, Carbohydrates, Terpenoids and Saponins by the standard identification methods and reagents.

2.5 Quantitative phytochemical screening

2.5.1 Alkaloids Determination

Alkaloids were quantified by using the protocol provided by Harborne (1973). Total 5 g sample was mixed thoroughly in (10%) 200mL acetic acid formulated in ethanol. Covered the solution and leave it for 4 hours at room temperature. After wards, extract was filtered and left the filtrate to reduce it to one third to its actual volume on water bath. The concentrated solution of ammonium hydroxide was added till the precipitation. The precipitates were washed with 0.2M ammonium hydroxide. A piece of filter paper was weighed and was set in the funnel for filtration. During filtration the entire residue was left behind on filter paper. Then this residue was dried in oven at 50°C for absolute dryness and weighed again. Alkaloid was quantified from the difference of paper with residue and empty filter paper. Furthermore experiment was repeated thrice and the mean value was calculated.

2.5.2 Determination of Saponin

The protocol used in this study for the quantification of saponins was modified by Obadoni and Ochuko[16]. Total 20 g of powdered seed sample was dispersed in 200 mL of ethanol (20%) in a glass beaker. This mixture was left in the water bath and heated at 55°C for 4-5 hours with constant shaking. Filtered the suspension and re-extracted the residue with 200 mL of 20% ethanol. Evaporate the collected extract up to 40mL by heating at 90°C on water bath. Transfer the concentrated extract in a separating funnel and slowly added 20 mL of diethyl ether followed by shaking. As a result ether and aqueous layer were obtained, and the aqueous layer was shifted to another flask whereas the layer containing ether was thrown out. The purification process was repeated twice and added about 60 mL of n-butanol in the resultant concentrate. Two times washing of purified butanol extracts was done with 10 mL sodium chloride solution (5%). Layer having sodium chloride was discarded and the final extract was warmed slowly till evaporation. Afterwards the residue was desiccated in the oven to constant weight and percentage of saponin was calculated.

2.5.3 Flavonoids Quantification

Total 10 g of sample was suspended in methanol having (80%) at normal temperature. After filtration of extract, weight of crucible with solution was measured. Evaporate the solvent in the water bath at 60°C. After complete evaporation the extract material left were weighed and flavonoids content was determined and percentage was calculated[17].

2.5.4 Quantification of Phenolic Contents

In sample of aqueous extract, total phenolic content was assessed by Folin-Ciocalteu reagent[18]. Five gram of sample was weighed by electric balance and dissolved in distilled water (Final volume 200mL). Then this mixture was heated along with continuous shaking at 45°C for 15 minutes on hot plate and resultant solution was filtered. To 1 mL of plant extract, added in it 2 mL of 10% Folin-Ciocalteu reagent and 3mL of sodium carbonate solution (2%) and left it for 20 min at room temperature. Spectrophotometric analysis of the sample was done at 765nm whereas 1mg/mL of gallic acid was served as standard. Total content of polyphenol in plant extract was calculated using standard curve analysis with gallic acid as standard expressed as equivalent to mg/g of extracted plant material. And procedure was repeated for triplicate analysis.

2.5.5 Quantification of Tannins

Quantification of tannins was carried out by Folin Ciocalteu method. 0.125g of dried plant sample was extracted with 25mL of 70% acetone in mechanical shaker for 12 hrs at 60°C. The mixture was filtered by Whatman filter paper no.42 and added 0.5 mL of Folin Ciocalteu reagent as well as 2.5 mL of Na_2CO_3 in filtrate. Spectrophotometric analysis was done at the wavelength of 725nm as compared to blank. Tannic acid in plant extract was calculated using standard curve results prepared with tannic acid which was used as standard expressed as mg/g of extracted compound. Experiment was repeated three times[19].

2.6 Quercetin estimation by spectrophotometer

Quercetin was quantified by the modified protocol given by Patil[20]. 100 mg of grounded sample was placed in a volumetric flask and 80% ethanol was added in it, placed on shaker for complete extraction. Then this flask was placed in sonicator for about 10 minutes, followed by filtration of the sample with the help of Whatman filter paper (No. 42), filtrate was poured in small falcon tubes and then stored at -20 °C for further process.

Ethanolic extracts were thawed and vortexed for few minutes and absorbance was measured at 362nm via spectrophotometer. The standard used in the analysis was quercetin with different concentrations i.e. 0.2-0.8 mg/mL. The calibration curve was drawn and expressed as mg/g of extracted compound and experiment was repeated three times.

2.7 Assessment of antioxidant activity

2.7.1 DPPH Radical Scavenging Assay

The plant seed extract for free radical scavenging activity was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method by applying the modified protocol of Lim *et al* [21]. The reaction involved has

DPPH + (H-A) → DPPH-H + (A)
(Violet color) (Light yellow color)

The antioxidant component in our plant extract undergo a reaction with DPPH by donating free electron, and the stable free radical (DPPH) undergone the process of reduction and forms DPPH-H. This in turn, reduces the absorption from the DPPH radical to the reduced form. The strength of discoloration from violet to light yellow color describes the scavenging ability of entire antioxidant compounds in the form of its hydrogen donating capacity[22].

Different concentrations of the *E.sativa* seed extract (0.2, 0.4, 0.6 and 0.8 mg/mL) were formulated in triplicates. In this method, 0.135mM of DPPH was made ready in absolute methanol (98%), thereafter 1mL of this DPPH solution was taken in a test tube and 1mL of plant extract was mixed in it. Mixture was shaken or vortexed, allowed standing in the dark for half an hour in order to complete the reaction. The spectrophotometer was set on 517nm and the absorbance was measured. Decrease in absorbance indicated the increase in anti oxidant ability. The DPPH radical scavenging strength of plant seed extract was calculated by the equation given below:

$$\text{DPPH Scavenging effect (\%)} = \frac{\text{(Absorbance of control- Absorbance of sample)}}{\text{Absorbance of control}} \times 100$$

Where

Absorbance of sample = DPPH + aqueous seed extract
Absorbance of control = DPPH + methanol (absolute)

The 50% inhibition (IC50) was considered as the concentrations of samples that has the ability to inhibit 50% of scavenging activity of DPPH radicals and was calculated via dose inhibition curve[23].

2.8 Extraction of oil through soxhlet

About 10g of moisture free powder for sample was placed in thimble and thimble was then placed in the soxhlet's loading chamber. The round bottom flask was filled with 300 mL of n-hexane. The temperature was set at medium mode of temperature. The solvent placed in the flask were boiled and vaporized. The whole process was repeated many times to ensure the complete oil extraction. The solvent turned into colorless, indicating the end point of the process. The oil was purified by placing it in rotary evaporator as a result the oil got free from the solvent. The purified oil was stored in sterilized and labeled falcon tubes[24].

2.9 Physiochemical properties of seed oil

Peroxide Value, acid Value, saponification Number, specific Gravity, pH of Oil and wax content were measured as per standard methods.

3. Results and Discussion

The present study was carried out to assess bioactivity of *Eruca sativa* seeds along with physiochemical analysis of seed extracts. Anti inflammatory and skin antiallergic activity was studied and the results are given in the following section.

3.1 Proximate analysis of plant sample

Proximate analysis of *Eruca sativa* seeds indicates the moisture content, crude protein, carbohydrate, crude fat and ash content Table 3.1. Based on these results *Eruca sativa* seeds provides good source of protein (30±1.2) and oil (29±1.6) as compared to other components. Previous study had shown that seeds of *Eruca sativa* possess moisture content 4.1%, ash 6.6%, crude protein level 27.4% and oil content was found to be 27.8%, however, oil contents in seeds depend on many factors including maturity of the seed as well as degree of plant irrigation[5]. Similar results were proposed by Asmma (2013) which revealed that proximate composition of *Eruca sativa* seeds contain moisture 6.6%, crude proteins 25.17%, and crude fiber 2.4% and total minerals ash 9.1%. Therefore results about proximate analysis of seeds are in closed agreement with the results reported by different authors who indicate results studied were valuable and indicate standard values of *E. sativa* varieties.

3.2Qualitative and quantitative estimation of phyto- chemicals from *Eruca sativa* seeds

Qualitative analysis of *Eruca sativa* seed indicated the presence of diverse plants metabolites such as alkaloids, phenolic acid, flavonoids, saponins and tannins (Table 3.2), whereas the quantification of phyto-constituent was mentioned in table 3.3.

Results depicts that *E. sativa* seed extracts possess significant amount of flavonoids (8.35±0.64), Phenols (14.13±1.40), alkaloids (5.38±0.004), saponins (0.66±0.26) and are highly rich in tannins (52.98±4.29). Phytochemical study of seed of *E. sativa* showed presence of all essential phyto constituents required for potential traditional medicine[25-26.] *Eruca* seed extract contains important secondary metabolite such as flavonoids, alkaloids, tannins, phenols, saponins, ascorbic acid and those are used as remedies of many diseases and frequently required in traditional medicines[8]. Inspite of that, it is mentioned in traditional pharmacopoeia and ancient literature that it does hold a numerous health promoting chemical agents comprising carotenoids, vitamin C, fibers, and glucosinolates (GLs) used for several therapeutic properties. The presence of large amount of these phytochemicals bestows high medicinal activities including antioxidant and anti allergic[27].

3.3 Quercetin determination in *Eruca Sativa* seeds

The concentration of Quercetin in *Eruca sativa* seeds was found to be 0.03326±0.002 ($y=32.58x$, $R^2=0.971$) using UV/Vis spectrophotometric analysis as shown in fig 3.2. Quercetin which is a typical example of the flavonol subclass is used as a nutritional supplement found in fruits and vegetables. It has the capacity to stop the oxidation of LDL by scavenging free radicals and chelating the transition metal ions. The quercetin may assist to prevent several diseases, such as atherosclerosis, cancer and chronic inflammation by a mechanism which retards oxidative degradation[28].

3.4 Determination of antioxidant activity of *Eruca sativa* seeds

For the assessment of plant extracts for antioxidant activity, the DPPH scavenging protocol is quite easy and sensitive**.** Numerous methods are employed for the evaluation of free radical scavenging assay but the stable 2, 2-diphenyl-1-picryl-hydrazyl radical (DPPH) is considered more important due to its availability and ease.

The antioxidant assay of the aqueous extract of *Eruca sativa* seed was investigated using DPPH scavenging assay. The ability of DPPH to decolorize a free radical depends upon the presence of antioxidant. The DPPH is a stable radical, having an odd electron which shows maximum absorbance at 517 nm. By accepting an electron by DPPH, provided by antioxidant compound, it decolorizes the DPPH which is calculated quantitatively by the change in absorbance of solution. The plant extract exhibited a significant dose dependent inhibition of DPPH activity with a 50% inhibition at a concentration of as compared with reference standard gallic acid which was also reported by Goyal[29].

Scavenging activity of plant extract is a function of its concentration, as the concentration of compounds to be tested raises; there is an increase in total radical scavenging where as lower IC50 value of any extract reflects improved protective action[30]. IC50 and anti radical power **(**ARP) for DPPH radical- scavenging activity was calculated graphically by using standard curve ($y=0.079$, $R^2 = 0.959$) for *Eruca* seeds and ($y = 0.095x$, $R^2 = 0.974$) for gallic acid. IC50 value of *Eruca sativa* was 126.2 µg/mL, with reference to ($y=0.079x$, $R^2=0.959$ (Fig 3.1).

The IC50 was calculated for *Eruca* seeds and that of gallic acid (standard) are enlisted in table 3.5. By changing the concentrations of test compounds scavenging effect was increased. The IC50 value for seed extract was comparatively higher than the IC50 of gallic acid (126.2µg).

DPPH radical scavenging activity of the extracts expressed as IC50 garden rocket was previously studied which showed that ethanolic extract showed high antioxidant activity (1.0 mg/mL) where as aqueous extract exhibits moderate DPPH radical scavenging activity of (3.1 mg/mL) as reported by Ismail *et al*[31].

Table 3.1: Proximate analysis (%) of *Eruca sativa* seeds

***E. sativa* seeds**	**Dry Matter**	**Moisture content**	**Ash content**	**Crude Fiber**	**Crude Protein**	**Crude Fat content**	**Carbohydrate content**
Sample I	94.5±2.5	5.5±0.5	2.9±0.6	1.4±0.3	30±1.2	29±1.6	3.55±0.6
Sample II	92.5±2.2	5.1±0.5	2.5±0.5	1.3±0.2	29±1.1	28±1.5	3.45±0.2
Sample III	93.5±1.8	5.9±0.5	2.3±0.4	1.4±0.3	27±1.0	26±1.2	3.33±0.4

Values are expressed in terms of Mean ±SD after triplicate analysis

Table 3.2: Qualitative analysis of phytochemicals from *Eruca sativa* seeds

S. No	**Phytochemicals**	***E. Sativa***		
		I	**II**	**III**
1	Alkaloids	+	+	+
2	Flavonoids	+	+	-
3	Phenols and tannins	+	+	+
4	Saponin	+	+	+
5	Terpenoids	+	+	+
6	Carbohydrates	+	+	-
7	Protein and amino acids	+	+	+
8	Reducing sugars	+	+	+
9	Cardiac glycoside	+	+	+
10	Steroids	+	-	-

Triplicate analysis (n=3)

Distilled water =I, ethanol=II. Methanol=III. Present +, Absent -, (n=3)

Table 3.3: Quantitative analysis of phytochemicals from plant extracts

***Eruca sativa* seeds**	**Flavonoids %**	**Alkaloids %**	**Saponin %**	**Tannins %**	**Phenols %**
Sample I	8.35±0.64	5.38±0.004	0.66±0.26	52.98±4.33	14.13±1.40
Sample II	8.05±0.54	5.18±0.05	0.58±0.23	50.98±2.29	13.99±1.29
Sample III	8.01±0.74	5.08±0.19	0.61±0.56	49.98±1.89	14.93±1.31

Values are shown in form of Mean±SD after triplicate analysis

Fig 3.1: Quercetin determination in *Eruca sativa* seeds

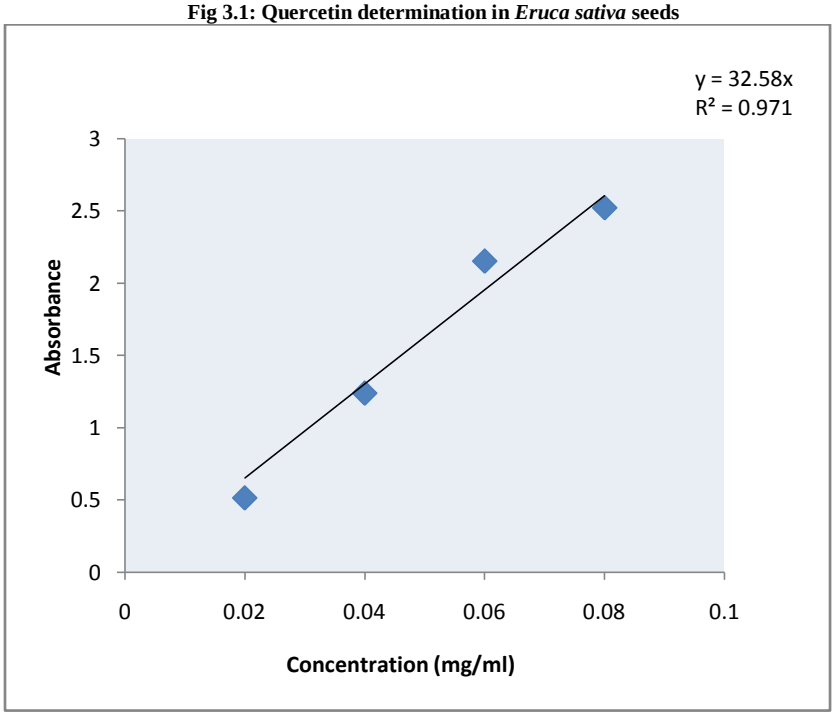

Table 3.4: DPPH Radical Scavenging assay of aqueous extract of *Eruca sativa* seeds

TEST	DPPH Free Radical Scavenging Activity (%age)	
Seed Extracts (µg/mL)	***Eruca Sativa* Seeds**	**Gallic acid (Standard)**
20µg/mL	10.37±0.04	11.0±0.1
40 µg/mL	14.72±0.07	16.26±0.3
60 µg/mL	25.22±0.3	30.0±0.6
80 µg/mL	30.60±1.1	38.54±1.0

Values are shown in the form of mean±SD after triplicate analysis

Fig 3.2: DPPH Free Radical Scavenging Activity of aqueous extract of *Eruca sativa* seed

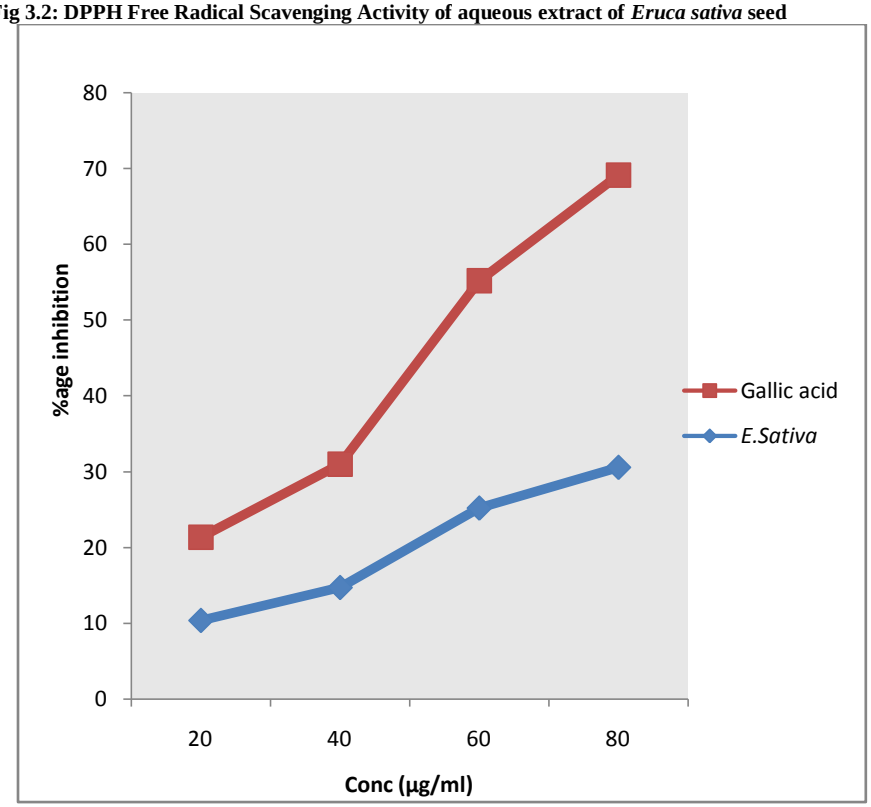

Table 3.5: IC_{50} and anti radical power (ARP) of aqueous seed extract and gallic acid

Sample	IC_{50}(µg)	ARP
Gallic acid	104.38	9.58×10^{-3}
Eruca sativa Seed	126.2	7.92×10^{-3}

Table 3.6: Physiochemical Properties of *Eruca sativa* seed oil

Oil Parameters	Values
Acid value	0.338±0.0062 mg KOH/g oil
Saponification value	174.43±3.03 g of NaOH/100 g oil
Peroxide value	6.66±1.527 meq 2/kg oil
Wax contents	17.073±0.050%
Specific gravity(30 ºC)	0.8005±0.000361
pH	7.103±0.015
Color	Dark Yellow
State at room temperature	Liquid

Values expressed in term of mean ±standard deviation after triplicate analysis

3.5 Physiochemical properties of *Eruca sativa* oil

Physiochemical parameters of oil were determined which are given in table 4.6. The Eruca seed oil was greenish brown in color, liquid at room temperature and even in a refrigerator. It was found that the specific gravity of *Eruca* oil was lower than values reported by Flanders and Abdulkarim[5]. According to Rudan and Klofutar. (1999), specific gravity of oils (vegetable) at any specific temperature when compared to water rises as the average molecular mass decreases[32].

Saponification number is an indicator of the average molecular weight and chain length which is inversely proportional to the molecular weight of the lipid[33]. The saponification number of the *Eruca* oil was found to be 174.43g NaOH/100g oil which is closed to saponification value of 168.1% reported by Flanders and Abdulkarim[5]. Lower values of saponification prove that they have greater molecular mass comparing to that of common oils. This parameter is dependent upon the extent of unsaturation which describes their iodine number along with the free fatty acid value[34].

The *Eruca* seed oil had a wax content of 2.14±0.023% as shown in table 4.6. Waxes are high melting- point 25esters of long chain carboxylic acids and long-chain alcohols. During the refining process, wax was removed from crude oils to clarify the oil. They have intense applications in cosmetic and pharmaceutical industry, lubricants, food products and polymers as well.

The peroxide value of *Eruca sativa* seed oil was found to be 6.66±1.527 meq 2/kg oil as given in table 3.6. In order to determine quality of fats and oils, this parameter is highly significant because it suggests the oxidative constancy of the oil for the period of storage. Fat or oil which is processed from premium quality oil seed kernels produces the new peroxide values to visualize like the oil[35].

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