IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
May 2017 Vol.:6, Issue:3
© All rights are reserved by M.S. El-Haysha et al.

Antioxidant Activity Studies on Extracts of *Eruca sativa* Seed Meal and Oil, Detoxification, the Role of Antioxidants in the Resistant Microbes

H.A. El-Fadaly,[1] S.M. El-Kadi,[1] M.M. El-Moghazy,[2] A.A.M. Soliman,[3] and M.S. El-Haysha*[3]

[1] *Agriculture Microbiology Department, Faculty of Agriculture, Damietta University, 34517, Damietta, Egypt.*

[2] *Animal Production Department, Faculty of Agriculture, Damietta University, 34517, Damietta, Egypt.*

[3] *Department of Animal Nutrition Research - Animal Production Research Institute (APRI) - Agricultural Research Center (ARC), Dokki, Giza, Egypt.*

Submission: 2 May 2017
Accepted: 7 May 2017
Published: 25 May 2017

www.ijsrm.humanjournals.com

Keywords: *Eruca sativa*, Plant extracts, Antioxidant, Total polyphenols, Total flavonoids, Reducing power (FRAP), ABTS radical, DPPH radical, Detoxification

ABSTRACT

E. sativa is a member of the Brassicaceae, which is considered to be an important chemopreventive plant family. The total polyphenols and flavonoids contents of *E. sativa* meal and oil have the highest of total polyphenols and flavonoids contents, which were 49.77 mg GAE/g and 11.82 mg GAE/g, respectively. Antioxidant activity of methanolic, hexane and heat-methanolic extracts and oil of *E. sativa* were determined by using FRAP, ABTS, and DPPH. The methanolic extract meal and oil of *E. sativa* have the highest reducing power which was ranged from 0.8925 to 2.0191 and 0.7596 to 1.4765 at the concentrations of 10 and 80 mg/ml, respectively. Also, by used (ABTS) it could be percentage were 72.19% (methanolic extract) and 65.23% (oil), While, by used (DPPH) it could be percentage of inhibition (IC_{50}) which were 0.059 to 0.041 for methanolic extract and 0.047 to 0.025 for oil at the concentrations of 250 and 1000 μg/ml, respectively.

INTRODUCTION

Soybean feed is considered the main plant of protein source in rabbit regimes. It is expensive in comparison to other non-traditional plant protein sources. These sources are an essential treat to enhance animal health and fertility. Few studies were carried out on rabbit non-conventional protein meal and literature available is not enough to overcome the nutritive issue and its effects on animal reproduction and health. They also contain (Mg, Mn, Zn, Cu, Fe) and other elements (Abdo, 2003), which increase immune response and the reproductive implementation. Carotenoids can shield phagocytic cells from antioxidative damage, enhance T and B lymphocyte proliferative rejoinders and increase the production of certain interleukins (Bendich, 1989). Also, they increase plasma I gG concentration (Chew *et al*., 2000). As said by World Health Organization (WHO), more than 80% of the world's population still relies on old-fashioned medicines for their basic health needs. Medicinal plants involvement in disease prevention and control has been attributed to antioxidant properties of their constituents, broadly termed as polyphenolic compounds. Along with their role as antioxidants, these compounds retains a broad spectrum of medicinal properties, for example, anti-inflammatory, antithrombotic, cardio-protective, anti-microbial, and vasodilatory (Demiray *et al.*, 2009).

HUMAN

Scientists are taking a keen interest in relating phytochemical constituents with their pharmacological actions. Phytochemicals such as flavonoids, terpenoids, lignans, glycosides and various alkaloids are well discrete in the plant kingdom and analyzed scientifically for its antiallergic movement (Koyama *et al.*, 2006 and Zheng *et al.*, 2009). Production of rocket (*Eruca sativa*) meal in Egypt has been steadily increased for the strong demand for volatile oils for pharmaceutical purpose. These plants were discovered to incarnate natural substances that promote health and ameliorate the body condition to counteract the stress of infection (Eisenberg *et al*., 1993).

The rocket seeds contain carotenoids, vitamin C, flavonoids, for instance, appiin and luteolin and glucosinolates the forerunners of isothiocyanates and sulforaphane (Talalay and Fahey, 2001), volatile oils like myristicin, apiole, and β- phellandrene (Bradley, 1992; Leung and Foster, 1996).

Eruca sativa nearby known as Taramira is grown in contradictory parts of Indo-Pak subcontinent. It has been refined since ancient times in Central Asia and Southern Europe. *Eruca*

sativa Mill usually mentioned to as “rocket salad, arugula, roquette, or silvery pepper” in English is a member of Brassicaceae family. It is minor oil crop and used in old-style medicines as remedies for different diseases. Its oil is mostly used in commerce in soap making, as an illuminating and lubricating agent, in massage and in medicines additionally (Ugur *et al.*, 2010). The extracted oil from the seed of *E. sativa* is forbidden for eating purposes because of its pungent and obnoxious odor. The cake is used as dung for improving the soil physical complaint fertility and can also be used as nutritional feed for animals (Mohammad *et al.*, 2009).

E. sativa seed extract contains significant subordinate metabolite such as flavonoids, alkaloids, tannins, phenols, saponins, ascorbic acid and those are used as remedies for many diseases and regularly required in old-fashioned medicines. Essential oil especially erucic acids were contemporary in high concentration those are responsible for the antibacterial activity, which could be used for the preparation of painkillers compulsory for human and animal health (Alam *et al.*, 2007). In outdated medicines, rocket species are documented for their therapeutic possessions such as astringent, digestive, diuretics, tonic, laxatives, rubefacient and stimulants etc. Rocket salad types are rich in antioxidant complexes, as a source of vitamins like ascorbic acid, carotenoids as well as polyphenols (Michael *et al.*, 2011). Glucosinolates were institute to have several biological activities including anticarcinogenic, antifungal, antibacterial plus their antioxidant accomplishment (Kim *et al*., 2004). The main glucosinolate in seeds is Erucin, which is hypothetically capable of protecting cells against oxidative stress via three instruments: (i) initiation of phase II enzymes, (ii) rummaging hydrogen peroxide and alkyl hydroperoxides accumulated in cells and peripheral blood and (iii) acting as a precursor of sulforaphane, a potent inducers detoxifying electrophiles and increase cellular antioxidant fortifications (Barillari *et al*., 2005).

Projected that *Eruca sativa* seeds exhibit antidiabetic effect by reducing oxidative stress investigated in rats. In addition, ethanolic extract of *Eruca sativa* possesses substantial anti-secretary, cytoprotective, and antiulcer activities against gastric lesions experimentally induced in rats by inspiring mucus synthesis and endogenous prostaglandins through its effective antioxidant activity (Alqasoumi *et al.*, 2009). Although, Mradu *et al.,* (2012), quantified that anti-oxidative activity is a measure of the capability of compound to scavenge free Hydroxyl groups and Oxygen species. It is the very imperative property of medicinal plants because there

is a number of intelligence which mentions that in biological systems, free radicals are causative agents for different disease such as cancer. Consequently, antioxidant properties are an index of antioxidant potential against sensitive oxygen species (free radicals).

Laterally with the other medicinal plants, *Eruca sativa* has remarkably probable against various diseases. Present work is created on the estimation of antioxidant potential of it. Water, ethanolic and methanolic extracts of *E. sativa* seed oil. On phytochemical determination, it was exposed that dissimilar plant extracts/fractions contain alkaloids, flavonoids, saponins, tannins, phenols, carbohydrates, steroids, and proteins. Its physicochemical screening was decided the saponification value and fat value of the *E. sativa* seed oil. Antioxidant activity was assessed by using DPPH free radical scavenging prearrangement. The maximum (30.60±1.1) anti-oxidative potential was revealed by water extract of *E. sativa* seed oil. IC_{50} was intended maximum 126.2 in eq. extract of *E. sativa* seed oil. From current study, it is decided that this plant seed oil could be used as a source of normal antioxidant and medicinal commitments (Hamid *et al.*, 2014).

Present study focus on the effective antioxidant activity of methanol (ME), Hexane (HE), methanolic extract after heat treatment (MHE) of *E. sativa* seed meal and *E. sativa* seed oil along with their antioxidant power. Total polyphenols and total flavonoids were determined and reducing power, antioxidant activities using ABTS and DPPH assays were tested.

MATERIALS AND METHODS

Preparation and analytical procedures of plant extracts

Sample meal and oil were conveyed directly to the laboratory of Agricultural Biochemistry at the Faculty of Agriculture, Mansoura University, Mansoura, Egypt. Flowers sample from each species was air dried in the shade and ground into a fine powder. The powdered air dried Flowers were divided into three extracts:

First extract: Powdered air dried meal (2 Kg) from each plant were extracted by soaking at room temperature for six times with methanol (30 L), then the methanolic extracts were concentrated to near dryness under reduced pressure using the rotary evaporator at 45°C to achieve the crude methanolic extracts which kept for further investigation (El-Khateeb *et al*., 2014).

Second extract: 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 hexane) 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 a hot plate till evaporation.

Extracts prepared were analyzed for the screening of bioactive compounds by standard methods (Sofowora, 1993; Trease and Evans, 1989 and Harborne, 1973).

Glucosinolates detoxification

Various treatment methods were tried to remove or reduce Gls content and to minimize Gls-associated deleterious effects on animal health and production. Most of these methodologies included hydrolysis or decomposition of glucosinolate before feeding. Chemical treatment and or supplementation were also tried to overcome glucosinolate related toxicity in animals (Tripathi and Mishra, 2007).

Detoxification Procedures

The following treatments were carried out according to Aregheore*et al.,* (2003) with some modifications:

Moist Heat Treatment+ Methanol

In order to detoxify the cake, a number of physical and chemical methods were employed. Soaking (3, 6 and 12h), steaming (30 and 60 min), boiling (30 and 60 min), autoclaving(15 psi, 30min; 15 psi, 60 min) and heating (100°C 30 min; 120°C 25 min) were the physical methods employed. Of all the methods employed, autoclaving (15 psi. for 60 min) and lime treatment (40 g/kg) completely destroyed the toxin (Anandan *et al.,* 2005).

Drastic heating (above 110°C, more than 30 min) of RSM impairs protein quality in non-ruminant's animals (Newkirk *et al.*, 2003; Glencross *et al.,* 2004).

For non-ruminant feeding, heating of RSM should be restricted to 30 min at 100ºC (Jensen *et al.,* 1995).

Approximately, 300 g of the defatted meal was mixed with distilled water to 66% moisture. The mixture was made into a paste, covered with aluminum foil and placed in an autoclave at 121^0C for 30 minutes. The autoclaved sample was dried at 25^0C for 5 hrs in a fan oven apparatus. It was milled to produce the meal (Azzaz *et al.*, 2011).

Hexane extraction treatment

25g portions of the powdered meal were each separately dispersed in 250ml of hexane. The solution was left to stand at room temperature for 24 hrs and was filtered with Whatman No. 1 filter paper. The filtrate was used for the phytochemical screening using the following tests. The results also show that sterols and terpenoids were more readily extracted by hexane but absent in water (Ugochukwu *et al*., 2013).

The purpose of the extraction hexane; Plant crude extracts usually contain large amounts of carbohydrates and/or lipoidal material and the concentration of the phenolic in the crude extract may be low. To concentrate and obtain polyphenol-rich fractions before analysis, strategies including sequential extraction or liquid-liquid partitioning and/or solid phase extraction (SPE) based on polarity and acidity have been commonly used. In general, elimination of lipoidal material can be achieved by washing the crude extract with non-polar solvents such as hexane (Ramirez-Coronel *et al*., 2004).

Determination of Antioxidant Factors

Total polyphenols content of taramira (Eruca sativa) meal and oil

Total phenolic contents of the air dried meal and oil were determined by using **Folin–Ciocalteu** reagent method according to Lin and Tang (2007), the following experiment has been achieved at Chemistry Department Faculty of Agriculture, Mansoura University. About 0.1g of air dried Flowers was dissolved, separately in 1 ml distilled water. Aliquots of 0.1 ml of the previous solution were taken and mixed with exactly 2.8 ml of distilled water, 2.0 ml of 2% (w/v) sodium carbonate and finally, 0.1 ml of 50% (v/v) of Folin–Ciocalteu reagent was added. The mixture was incubated for 30 minutes at room temperature and the absorbance of the resulting color was measured at 750 nm against distilled water as blank, using a Spell 11 (Carl Zeiss-Jena) spectrophotometer. For quantitative determination, a standard curve of gallic acid (0-200mg/l)

was prepared in the same manner. Total phenol contents were expressed as milligram gallic acid equivalent (GAE)/g based on dry weight (Fig. 1).

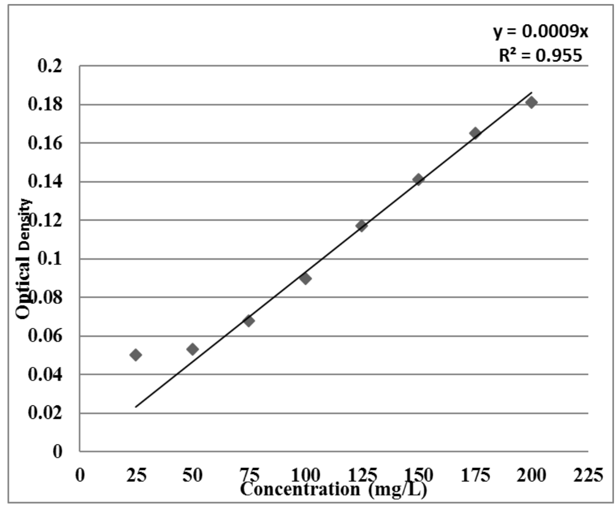


Fig. 1: Standard curve of gallic acid.

Total flavonoids content of taramira meal and oil

Flavonoids are polyphenolic compounds comprising fifteen carbons with two aromatic rings connected by a three-carbon bridge, hence C6-C3-C6 (Fig. 2). They are the most numerous of the phenolics and are found throughout the plant kingdom (Crozier *et al.*, 2006 and Pereira *et al.*, 2009). They are present in high concentrations in the epidermis of leaves and fruits and have important and varied roles as secondary metabolites, being involved in processes like UV protection, pigmentation, stimulation of nitrogen-fixing nodules and disease resistance (Crozier *et al.*, 2006 and Pereira *et al.*, 2009). Flavonols are the most widespread of the flavonoids. quercetin, kaempferol and isorhamnetin, the main flavonols in Brassica crops, are most commonly found as O-glycosides. Conjugation occurs most frequently at the 3 position of the C-ring, but substitutions can also occur at the 5, 7, 4´, 3´ and 5´ positions (Hollman and Arts 2000; Crozier *et al.*, 2006; and Aronand Kennedy 2008).

Quercetin

Kaempferol

Isorhamnetin

Cyanidin

Fig. (2): Flavonoid aglycones found in vegetable *Brassica* crops.

Total flavonoids content of the air dried meal and oil were determined colorimetrically using aluminum chloride as described by Chang *et al.*, (2002), the following experiment has been achieved at Chemistry department Faculty of Agriculture, Mansoura University. About of0.1g of air dried taramira (*Eruca sativa*) meal and oil were dissolved in 1ml of distilled water. The resulting solution (0.5 ml) was mixed with 1.5 ml of 95% ethyl alcohol, 0.1 ml of 10% aluminum chloride ($AlCl_3$), 0.1ml of 1M potassium acetate (CH_3COOK) and 2.8 ml of distilled water. After incubation at room temperature for 40 min, the reaction mixture absorbance was measured at 415nm against distilled water as blank, using a Spell 11 (Carl Zeiss-Jena) spectrophotometer. Quercetin was chosen as a standard of flavonoids for making the standard curve (0–50mg/l). The concentration of total flavonoids contents was expressed as milligram quercetin equivalent (QE)/g based on dry weight (Fig. 3).

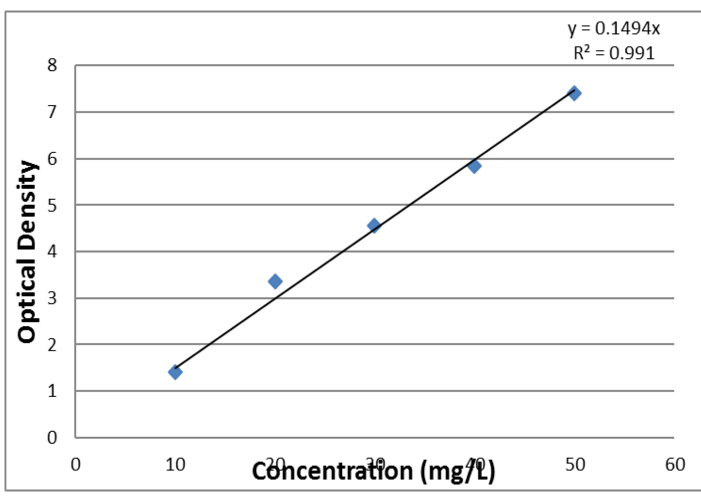


Fig. (3): Standard curve of quercetin.

Reducing power of methanolic and hexanoic meal extract and oil as absorbency of reactant

Reducing power of methanolic flowers extracts was determined according to the method of Oyaizu (1986), The following experiment has been achieved at Chemistry department Faculty of Agriculture, Mansoura University: Extract (0–100 mg) from each sample in 0.20mol phosphate buffer, pH 6.6 (2.5 ml) was added to 2.5ml potassium ferricyanide (10 mg/ml), mixture was incubated at 50 ^{o}C for 20 min. Trichloroacetic acid (TCA) (2.5 ml, 100 mg/ml), was added to the mixture then centrifuged at 650g for 10 minutes. The supernatant (2.5 ml) was mixed with distilled water (2.5ml) and 0.5ml ferric chloride solution (1 mg/ml) was added and the absorbance of the resultant color was measured using a Spell 11 (Carl Zeiss-Jena) spectrophotometer at 700nm. The Higher absorbance of the reaction mixture indicated greater reducing power.

Determination of ABTS radical scavenging activity

ABTS (2,2'-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) assay was based on the method of Re *et al.,* (1999) with slight modifications. 2 ml of ABTS solution (1 mg/1 ml 0.1M phosphate buffer, pH 7.0) were added to 3 ml of MnO_2 (25 mg/ml in previous phosphate buffer). The mixture was shaken and centrifuged for 10 minutes, clear supernatant was separated.

Exactly 1mg of a crude methanolic meal was dissolved in a mixture solvent (1ml) of methanol and previous phosphate buffer in the ratio of 1:1. Resultant extract solution (20 µl) was added to

the ABTS solution mixture, as previously described. A positive control sample was prepared exactly in the same manner but differ only in the addition of 20µl of 2mM ascorbic acid, instead of extract solution. A blank sample was prepared exactly in the same manner but differ only in the addition of 20µl of distilled water, instead of extract solution.

The absorbance of the resulting greenish-blue solution was recorded at wavelength 734nm, using a Spell 11 (Carl Zeiss-Jena) spectrophotometer. The decrease in absorbance is expressed as a percentage of inhibition which was calculated from the following equation:

$$\text{Percentage inhibition } (\%) = (A_{Blank} - A_{Test}) / A_{Blank} \times 100 \quad \textbf{Eq. (1)}$$

Determination of (DPPH) radical scavenging activity

The DPPH free radical scavenging activity of Asteraceae *(Family Compositae)* flowers extracts at different concentrations were measured from bleaching of the purple color of (2.2 Diphenyl -1-picryl hydroxyl) *assay* was based on the method of Pratap *et al.,* (2013). A 0.1 ml solution of a different concentration of extract was added to 1.4 ml of DPPH and kept in dark for 30 min. The absorbance was measured at 517 nm, using a Spell 11 (Carl Zeiss-Jena) spectrophotometer. And the percentage inhibition was calculated by using Eq. (1).

RESULTS AND DISCUSSIONS

Total polyphenols and total flavonoids content of investigated flowers

Total polyphenols include several classes of phenolic compounds that are secondary plant metabolites and integral part of human and animal diets. Flavonoids are a large group of the phenolic compounds consisting mainly of flavonols, flavanols, and anthocyanins. Phenolic compounds can play an important role in preventing body cells and organs from injuries by hydrogen peroxide, damage by lipid peroxides and scavenging or neutralizing free radicals (Sroka and Cisowski, 2003).

It has been reported that free radical scavenging and antioxidant activity of many medicinal plants are responsible for their therapeutic effect against cancer, diabetes, tissue inflammatory and cardiovascular diseases (Cai *et al.,* 2004). Also, it was found that high total phenols content

increase antioxidant activity and there is a linear correlation between phenolic content and antioxidant activity in fig leaves extract (Changwei *et al.,* 2008).

Table (1) and Fig. (4a) showed the total polyphenols (mgGAE/g) and total flavonoids (mgQE/g) contents of *Eruca sativa* meal extract and oil.

It was clear that, *Eruca sativa* meal (Methanol extract without thermal treatment), oil have the highest concentration of total polyphenols, was 49.77 and 44.22 mg GAE/g, respectively. While meal with heat treatment has the low concentration of total polyphenols, was 26.11mg GAE/g. Also, Hexane extracts of *E. sativa meal* containing medium values of total polyphenols which were 31.44 mg GAE/g.

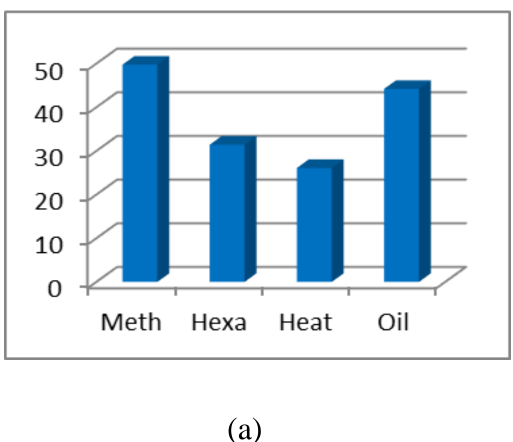
Total flavonoids as shown in Table (1) and Fig. (4b) ranged from 4.59 to 11.82 mgQE/g dry weight for Heat treatment and Methanol extract of *E. sativa* meal, respectively.

Lamy *et al.* (2008) provided an assessment of the bioactive effects of rocket plant extract in a human cell culture system. This could help to evaluate the balance between beneficial vs. possible adverse effects of rocket plant consumption.

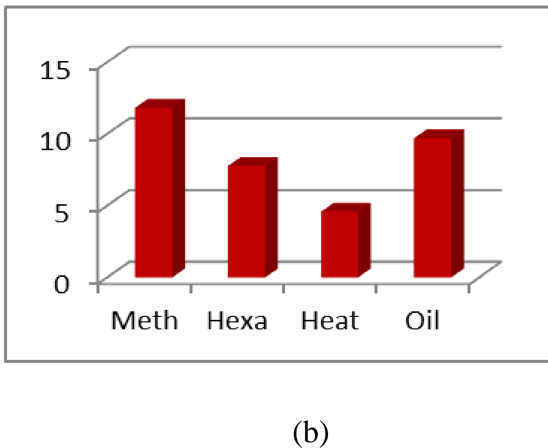
HUMAN

Table 1. Total polyphenols (mg GAE/g) and total flavonoids (mg QE/g) contents of Transactions.

Transactions	Total polyphenols (mgGAE/g)	Total flavonoids (mgQE/g)
Methanol extract	49.77	11.82
Hexane extract	31.44	7.77
Heat treatment	26.11	4.59
Oil	44.22	9.70



(a)



(b)

Fig. (4): (a) Total polyphenols (mgGAE/g) content of *E. sativa* meal extracts and oil. (b) Total flavonoid (mgQE/g) content of taramira meal extracts and oil.

The flavonoids and phenolic compounds in the plant have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc. (Lalitha and Jayanthi, 2012). (Jaiswal *et al.,* (2011), found that all the vegetable extracts had high flavonoid contents in the order of 21.7, 17.5, 15.4 and 8.75 mg QE/g of extract (DW) for York cabbage, broccoli, Brussels sprouts and white cabbage, respectively. York cabbage extract had the highest total phenolic content, which was 33.5, followed by 23.6, 20.4 and 18.4 mg GAE/g of dried weight (DW) of the extracts for broccoli, Brussels sprouts and white cabbage, respectively.

Reducing power (FRAP) of different of extracts as absorbency of reactant

The efficiency of methanolic flowers extracts to reduce Fe^{+++} to Fe^{++} was determined according to the method of Oyaizu (1986). The optical density of reaction mixture was measured at wavelength 700nm using a Spell 11 (Carl Zeiss-Jena) spectrophotometer. The obtained data are presented in table (2) as absorbency showed the reducing power for different of crude methanol, hexane and heat treatment extracts and oil of *E. sativa*. Data expressed as absorbance at 700nm for producing color as a result of using four concentrations (10, 20, 40, and 80 mg/ml) for each sample.

Table 2. Reducing power of transactions determined as absorbency at 700 nm.

Concentration mg/ml	Optical density at 700nm			
	Methanol extract	***Hexane extract***	***Heat treatment***	***Oil***
10	0.8925	0.5554	0.1570	0.7596
20	1.1921	0.7919	0.3219	0.9902
40	1.7902	0.9502	0.6518	1.2488
80	2.0191	1.1719	0.9409	1.4765

From table (2) and fig. (5), some points could be deduced: (1) The reducing power capacity increased with increasing the methanolic extract and oil concentrations for all samples. (2) *E. sativa* has the highest reducing power which was ranged from 0.8925 to 2.0191 for methanolic extract at the concentrations of 10 and 80 mg/ml, respectively. While, *oil* have the highest reducing power which was ranged from 0.7596 to 1.4765 at the concentrations of 10 and 80 mg/ml, respectively. (3) High levels of reducing power indicated the presence of some compounds which are electron donors and could react with free radicals to convert them into more stable products (Arabshahi and Urooj, 2007).

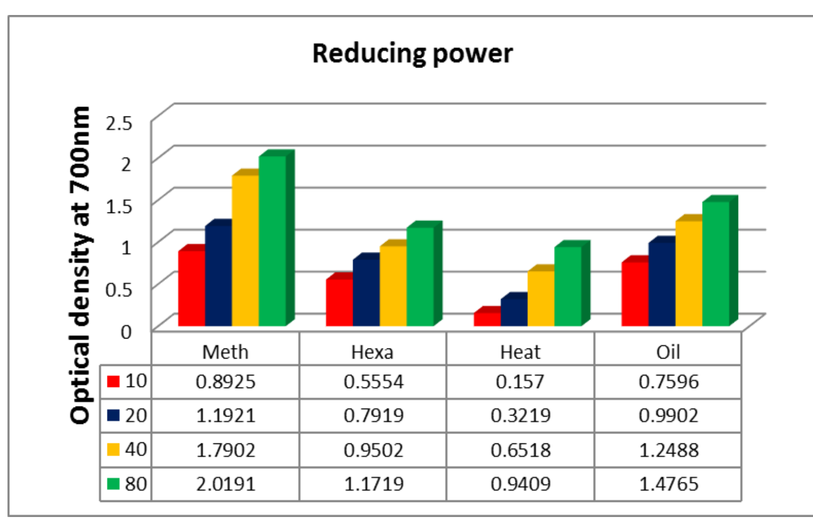


Fig (5): Reducing power of crude different extracts and oil determined as absorbency at 700 nm.

Determined by ($ABTS^+$) action radical

The capacity of methanol, hexane and heat treatment extracts meal and oil *E. sativa* to scavenge the ABTS radical was determined separately and compared with the reduction of ascorbic acid as a control sample which is known as a strong reducing agent (Fig. 6).

Fig. (6): Chemical structure of (2Z,2'Z)-2,2'-(hydrazine-1,2-diylidene)*bis*(3-ethyl-2,3-dihydro-benzo[*d*]thiazole-6-sulfonic acid) (ABTS).

From Table (3) and Fig. (7), it could be seen that all extracts showed different degrees of inhibition capacity, but their capacities were inferior to ascorbic acid which has the maximum inhibition (91.41%).

HUMAN

Moreover, the capacity of heat treatment, hexane extracts, oil, and methanol extract to scavenge the ABTS radical was 41.49, 59.67, 65.23 and 72.19%, respectively.

The seed oil of *E. sativa* has promising pharmacological efficacies and ensures the presence of bioactive components responsible for the observed beneficial effects. Our findings support its use in traditional medicine as antimicrobial bioagent and highlight the potential of this food plant for its possible clinical use (Khoobchandani *et al*., 2010).

Different studies indicated that the electron donation capacity which reflecting the reducing power of bioactive compounds was associated with the antioxidant activity (Siddhuraju *et al.*, 2002).

Table 3. Antioxidant capacity of transactions determined by ($ABTS^+$) action radical.

Transactions	Absorbance	% Inhibition
Methanol extract	0.099	72.19
Hexane extract	0.062	59.67
Heat treatment	0.092	41.49
Oil	0.069	65.23
+Ve Control (Ascorbic acid)	0.045	91.41
-Ve Control	0.524	0

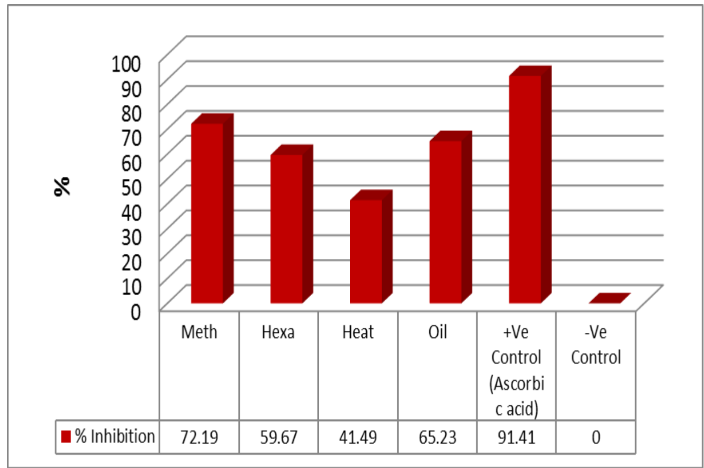


Fig (7): Antioxidant capacity of extracts determined by ($ABTS^+$) cation radical.

Determined by (DPPH) action radical

The capacity of *Cynara Cardunculus, Achillea Millefolium, Calendula Officinalis, and Matricaria Chamomillamethanolic* flowers extracts, radical scavenging ability using the stable radical DPPH (Fig. 8).

The antioxidant activity of the extracts prepared and oil from the studied plant species are reported in Table (4) and Fig (9).

Fig. (8): Molecular structure for DPPH.

The concentration of an antioxidant needed to decrease the initial DPPH concentration by 50% (IC_{50}) is a parameter widely adopted to measure the antioxidant activity (Sanchez *et al*., 1998). The lower IC_{50} pointed to the higher antioxidant activity.

The antioxidant activity of the tested extracts was measured using DPPH radical scavenging activity. The antioxidants scavenging activities for DPPH are attributed to their hydrogen-donating abilities (Biswas *et al.,* 2010). Vitamin C was used as the reference compound.

Table 4. Antioxidant capacity of Transactions determined by (DPPH) action radical.

Concentration µg/ml	IC_{50} (DPPH)			
	Methanol extract	*Hexane extract*	*Heat treatment*	*Oil*
250	0.059	0.031	0.012	0.047
500	0.053	0.027	0.009	0.038
1000	0.041	0.019	0.006	0.025

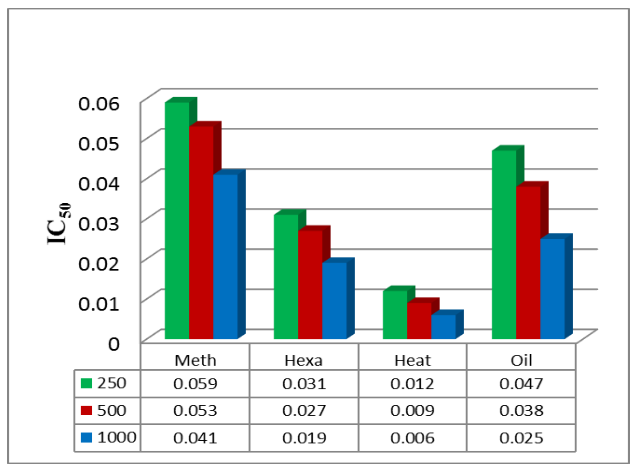


Fig. (9): Antioxidant capacity of extracts determined by DPPH cation radical.

Table (5): The effect of antioxidants on the antibacterial activity was determined against Gram-positive and Gram-negative bacteria

<table>
<tr><th>Natural antioxidant mechanism</th><th>Authors</th></tr>
<tr><td>All aerobic organisms, including human beings, have antioxidant defenses that protect against oxidative damage, and numerous damage removal and repair enzymes are present to either remove or repair damaged molecules.</td><td>Ali et al., 2001</td></tr>
<tr><td>York cabbage extract exhibited significantly higher antibacterial activity against Listeria monocytogenes (100%) and Salmonella abony (94.3%), being the most susceptible at a concentration of 2.8%, whereas broccoli, Brussels sprouts, and white cabbage had moderate to weak activity against all the test organisms.</td><td>Jaiswal et al., 2011</td></tr>
<tr><td>The antibacterial activity of the first three dilutions of cabbage extracts obtained from different concentrations of methanol (0, 20, 40, 60, 80 and 100%). Overall, the most susceptible among food pathogenic bacteria tested in this work to the antibacterial activity of cabbage extract was L.monocytogenes, followed by S. abony, and among the food spoilage, P. aeruginosa was more susceptible than E. faecalis.
<table>
<tr><th>Strain</th><th>the highest antibacterial activity</th></tr>
<tr><td>L. monocytogenes (100%)</td><td>60% methanolic extract</td></tr>
<tr><td>S. abony</td><td>60% methanolic extract</td></tr>
<tr><td>P. aeruginosa</td><td>60% methanolic extract</td></tr>
<tr><td>E. faecalis(62.9%)</td><td>80% methanolic extract</td></tr>
</table>
</td><td>Jaiswal et al., 2012</td></tr>
</table>

CONCLUSION

The medicinal plant extract containing a high percentage of active compounds such as polyphenols, flavonoids and glucosinolates, and their hydrolysis products for *E. sative* meal and oil belonging to the *Brassicaceae* family. The impact of natural extracts as antioxidant tested using (DPPH-ABTS-FRAB assays) showed the high ability of these plants to scavenging the free radicals in the laboratory, which may have antibacterial, antioxidant and anticancer properties.

ACKNOWLEDGMENT

We acknowledge El-Khateeb, Ayman Y., Assist. Professor of Agric. Biochemistry, Agricultural ChemistryDepartment, Faculty of Agriculture, Mansoura University, Egypt.

REFERENCES

1. Abdo, M.A. (2003). Using Egyptian *Eruca sativa* seed meal in broiler ration with or without microbial phytase. *Egypt J. Nutr. Feeds*, 6: 97–114.
2. Alam, M. S.; Kaur, G.; Jabbar, Z.; Javed, K. and Athar, M. (2007). *Eruca sativa* seeds possess antioxidant activity and exert a protective effect on mercuric chloride induced renal toxicity. *Food. Chem. Toxicology,* 45(6): 910–920.
3. Ali, Y., Mavi, A. and Kara, A.A. (2001). Determination of antioxidant and antimicrobial activities of *Rumex Crispus* L. extracts. *Journal of Agricultural and Food Chemistry, 49,* 4083-4089.
4. Alqasoumi, S.; Sohaibani, M. A.; Howiriny, T. A.; Yahya, M.A. and Rafatullah, S. (2009). Rocket "*Eruca sativa*"; A salad herb with potential gastric antiulcer activity. World J. Gasroenterol., 15(16): 1958-1965.
5. Anandan S. Anil Kumar G.K., Ghosh J. and Ramachandra K.S. (2005). Effect of different physical and chemical treatments on detoxification of ricin in castor cake. *AnimalFeed Science and Technology* 120: 159-168.
6. Arabshahi, D. S., and Urooj, A. (2007). Antioxidant properties of various solvent extracts of mulberry (*Morusindica*L.) leaves. Food Chemistry, 102, 1233–1240.
7. Aron, P.M. and Kennedy, J.A. (2008). Flavan-3-ols: Nature, occurrence and biological activity. *Mol. Nutr. Food Res.*, *52*, 79-104.
8. Azzaz, N.A.E.; El-Nisr, Neveen A.; Elsharkawy, Eman E., and Elmotleb, Eman A. (2011). Chemical and Pathological Evaluation of *JatrophaCurcas*Seed Meal Toxicity With or Without Heat and Chemical Treatment. Australian Journal of Basic and Applied Sciences, 5(12): 49-59.
9. Barillari, J.; Canistro, D.; Paolini, M.; Ferroni, F.; Pedulli, G.F.; Iori, R. and Valgimigli, L. (2005). Direct antioxidant activity of purified glucoerucin, the dietary secondary metabolite contained in rocket (*Eruca sativa* Mill) seeds and sprouts. *J. Agric. Food Chem*., 6: 2475–82.
10. Bendich, A. (1989). Carotenoids and the immune response. *J. Nutr*.,119: 112–5.
11. Biswas, M; Haldar, P. K., and Ghosh A. K. (2010). Antioxidant and free-radical-scavenging effects of fruits of *Dregeavolubilis.* J Nat SciBiol Med.; 1(1): 29–34.
12. Bradley, P.R. (1992). *British Herbal Compendium*, Vol. 1, Pp: 395–9. Bournemouth: British Herbal Medicine Association
13. Cai, Y.; Luo, Q.; Sun, M. and Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci., 74, 2157-2184.

14. Chang, C. C.; Yang, M. H.; Wen, H. M., and Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis, 10, 178–182.
15. Changwei, A. O.; Anping, L.; Abdelnaser, A.; Tran, D. X. and Shinkichi, T. (2008). Evaluation of antioxidant and antibacterial activities of Ficus macrocarpa L. leaf extract. Food Control, 19, 940-948.
16. Chew, B.P.; Park, J.S.; Wong, T.S.; Kim, H.W.; Weng, B.C.; Byrne, K.M.; Hayek, M.G. and Reinhart, G.A. (2000). Dietary β-carotene stimulates cell-mediated and humoral immune response in dogs. *J. Nutr.*, 130: 1910–3.
17. Crozier, A.; Jaganath, I.B. and Clifford, M.N. (2006). Phenols, polyphenols, and tannins: An overview. In *Plant Secondary Metabolites: Occurrence, Structure, and Role in the Human Diet*; Crozier, A., Clifford, M., Ashihara, H., Eds.; Blackwell: Oxford, UK, pp. 1-24.
18. Demiray, S.; pintado, M.E., and Castro, P.M.L. (2009). Evaluation of phenolic profile And antioxidant activities of Turkish medicinal plants: *Tiliaargentea, Crataegi folium* leaves, and *ploygonumbisto rta*roots. *World Acad. Sci. Eng. Technol*: 54.
19. Eisenberg, D.M.; Kessler, R.C.; Foster, C.; Norlock, F.E.; Calkins, D.R., and Delbanco, T.L. (1993). Unconventional medicine in the United States. Preference, cost, and patterns of use. *N. England J. Med.*, 328: 246–52.
20. El-Khateeb, A. Y; Azzaz, N. A., and Mahmoud, H. I. (2014). Phytochemical constituents, hypoglycemic and hematological effects of methanolic*Acalyphawilkesiana*leaves extract on streptozotocin-induced diabetic rats. European Journal of Chemistry, 5 (3) 430-438.
21. Glencross B, Hawkins W. and Curnow J. (2004). Nutritional assessment of Australian canola meal. Part II. Evaluation of canola oil extracted methods on the protein value of canola meal fed to the red seabream (*Pagrusauratus*, Paulin). *Aquaculture Research* 35: 25-34.
22. Hamid, S.; Sahar, A.; Malik, F.; Hussain, S.; Mahmood, R.; Ashfaq, K.M.; Malik, T.A.; Hassan, A. and Chaudhry, A.H. (2014). *Physico-chemical* investigation and antioxidant activity studies on extracts of *Eruca sativa* seed. *International Journal of Pharmaceutical Chemistry* (Online), American Chemical Society, 04 (04): 160-165.
23. Harborne, J. B. (1973). Phytochemicals Methods.Chapman and Hall Ltd., London, 49-188.
24. Hollman, P.C.H., and Arts, I.C.W. (2000).Flavonols, flavones and flavanols - nature, occurrence and dietary burden. *J. Sci. Food Agric.*, *80*, 1081-1093.
25. Jaiswal, A., Rajauria, G., Abu-Ghannam, A. and Gupta, S.(2011). Phenolic Composition, Antioxidant Capacity and Antibacterial Activity of Selected Irish Brassica Vegetables. NPC: Natural Product Communications, Vol. 6, no. 9, pp.1299-1304.
26. Jaiswal, A.K.; Rajauria, G.; Abu-ghannam, N. and Gupta, S. (2012). Effect of different solvents on polyphenolic content, antioxidant capacity and antibacterial activity of Irish York cabbage. *Journal of Food Biochemistry* 36: 344–358.
27. Khoobchandani, M.; Ojeswi, B.K.; Ganesh, N.; Srivastava, M.M.; Gabbanini, S.; Matera, R.; Iori, R. and Valgimigli, L. (2010). Antimicrobial properties and analytical profile of traditional*Eruca sativa* seed oil: Comparison with various aerial and root plant extracts. Food Chemistry, Volume 120, Issue 1, Pages 217–224.
28. Kim, S.J.; Jin, S. andIshii, G. (2004). Isolation and structural elucidation of 4-(B-d-lucopyranosyldi sulfanyl) butyl glucosinolate from leaves of rocket salad (*Eruca sativa* L.) and its antioxidative activity. *Biosci. Biotechnol*., 68: 2444–50.
29. Koyama J.; Morita, I.; Kobayashi, N.; Hirai, K.; Simamura, E.; Nobukawa, T. and Kadota, S. (2006). Antiallergic Activity of Aqueous Extracts andConstituents of TaxusYunnanensis. *Biol. Pharm. Bull.*, 29(11): 2310-2312.
30. Lalitha, T. P., and Jayanthi, P. (2012). Asian J. Plant Sci. Res., 2 (2): 115-122.
31. Lamy, E.; Schröder, J.; Paulus, S.; Brenk, P.; Stahl, T. and Mersch-Sundermann, V.(2008).Antigenotoxic properties of *Eruca sativa* (rocket plant), erucin and erysolin in human hepatoma (HepG2) cells towards benzo(*a*)pyrene and their mode of action. *Food and Chemical Toxicology*, 46, 7, 2415–2421.

32. Leung, A.Y. andFoster, S. (1996). *Drugs and Cosmetics*, 2nd Encyclopedia of common natural ingredients used in food. New York: John Wiley and Sons, Inc., USA.
33. Lin, J-Y. and Tang, C-Y. (2007). Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chemistry, 101, 140–147.
34. Michael, H.; Shafik, R. and Rasmy, G. (2011). Studies on the chemical constituents of the fresh leaf of *Erucasativa*extract and its biological activity as an anticancer agent *in vitro*. *J. Med. Plants. Res*., 5(7): 1184-1191.
35. Mohammad, H.; Chakrabarti and Rafiq, A. (2009). Investigating the possibility of using least desirable edible oil of *Eruca sativa* in Biodiesel Production*. Pak. J. Bot.,* 41: 481-487.
36. Mradu, G.; Saumyakanti, S.; Sohini, M. and Arup, M. (2012). HPLC Profiles of Standard Phenolic Compounds Present in Medicinal Plants. Int. J. PharmacogPhytochem, Res 4: 162-167.
37. Newkirk R.W., Classen H.L., Scott T.A. and Edney M.J. (2003). The digestibility and content of amino acid in toasted and non-toasted canola meal. *Canadian Journal of Animal Science* 83: 131-139.
38. Oyaizu, M. (1986). Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine, Jpn. J. Nutr., 44, 307–315.
39. Pereira, D.M.; Valentao, P.; Pereira, J.A. and Andrade, P.B. (2009). Phenolics: From Chemistry to Biology. *Molecules*, *14*, 2202-2211.
40. Pratap, C. R.; Vysakhi, M. V.; Manju, S.; Kannan, M.; Abdul, K. S., and Sreekumaran, N. A. (2013). In vitro free radical scavenging activity of Aqueous and Methanolic leaf extracts of *Aegletamilnadensis (Rutaceae).*Int J Pharm Sci, 819-823.
41. Ramirez-Coronel, M.A.; Marnet, N.; Kolli, V.S.; Roussos, S.; Guyot, S. and Augur, C. (2004). Characterization and estimation of proanthocyanidins and other phenolics in coffee pulp (Coffeaarabica) by thiolysis-high-performance liquid chromatography. *J. Agric. Food Chem.*, *52*, 1344-1349.
42. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M. and Rice-Evans C. (1999). Antioxidant activity applying an improved ABTS radical. cation decolorization assay. Free Radical Biology & Medicine, Vol. 26, 1231–1237.
43. Sanchez, M. C; Larrauri, A. and Saura, C. F. (1998). A procedure to measure the antiradical efficiency of polyphenols. Journal of the Science of Food and Agriculture, 76, 270–276.
44. Siddhuraju, P; Mohan, P. S., and Becker, K. (2002). Studies on the antioxidant activity of Indian laburnum (*Casia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers, and fruits pulp. Food chemistry, 79, 61-67.
45. Sofowora, A. (1993). Medicinal plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, 191-289.
46. Sroka, Z. and Cisowski, W. (2003). Hydrogen peroxide scavenging, antioxidant and antiradical activity of some phenolic acids. Food Chem. Toxicol., 41, 753-758.
47. Talalay, P. and Fahey, J.W. (2001). Phytochemicals from cruciferous plants protect against cancer by modulating carcinogen metabolism. *J. Nutr*., 131: 3027–33.
48. Trease, G.E., and Evans, W.C. (1989). Pharmacognosy, 11th edn.,Bailliere Tindall, London, 45-50.
49. Tripathi, M.K. and Mishra, A.S. (2007).Glucosinolates in animal nutrition: A review. Animal Feed Science and Technology 132: 1–27.
50. Ugochukwu, S. C.; Uche, A., and Ifeanyi, O. (2013). Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetiatripetala*G. Baker. Asian Journal of Plant Science and Research, 3(3):10-13.
51. Ugur, A.; Süntar, I.; Aslan, S.; Orhan, I. E.; Kartal, M.; Nazim, S.; Esiyok, D. and Sener, B. (2010). Variations in fatty acid compositions of the seed oil of *Eruca sativa* Mill caused by different sowing periods and nitrogen forms. Pharmacogn Mag., 6 (24): 305–308.

52. Zheng, C. J.; Tang, W. Z.; Huang, B. K.; Han, T.; Zhang, Q. Y.; Zhang, H. and Qin, L. P. (2009). Bioactivity-guided fractionation for analgesic properties and constituents of *Vitexnegundo* L. seeds. *J. Phytomed*., 16: 560-567.