Studies on Phytochemicals, Antioxidant, Free Radical Scavenging and Lipid Peroxidation Inhibitory effects of *Trachyspermum ammi* seeds

Vivek Kumar Bajpai[1,*], Pooja Agrawal[2]

[1]Department of Applied Microbiology and Biotechnology, Yeungnam University, Gyeongsan, Gyeongbook 712-749, Republic of Korea
[2]Madhya Pradesh Council of Science & Technology, Vigyan Bhawan, Bhopal 462-003 MP, India

ABSTRACT

Objective: Reactive oxygen species and free radicals generate oxidative stress in the living cells associated with number of chronic diseases. Current research is directed towards finding naturally occurring antioxidants of plant origin. Hence, this study was aimed to determine phytochemical analysis, antioxidant and free radical scavenging potential of ethanolic seed extract of *Trachyspermum ammi* (ESETA). **Methods:** A preliminary phytochemical screening was carried out for the analysis of important phytochemicals present in ESETA. Antioxidant potential of ESETA was evaluated by various scavenging models including DPPH, nitric oxide, superoxide, and hydroxyl radical as well as its lipid peroxidation ability in bovine brain extract. **Results:** The phytochemical analysis of ESETA revealed the presence of various phytoconstituents including alkaloids, glycosides, terpenoids, saponins, phenols and steroids. The ESETA showed the antioxidant capacity as the inhibition of DPPH radical by 73.41%. Also the ESETA had potent inhibitory effect on scavenging nitric oxide, superoxide, and hydroxyl radicals by 67.33%, 63.22% and 62.48% respectively. Moreover, the ESETA displayed concentration-dependent reducing power ability and remarkable ferric ion-induced lipid peroxidation inhibitory effect (69.22%) in bovine brain extract. **Conclusion:** These findings confirm the pharmacological efficacy of *T. ammi* as a potential source of natural antioxidant.

Key words: *Trachyspermum ammi*, Phytochemicals, Reactive oxygen species, Free radicals, Antioxidant activity.

INTRODUCTION

There has been strong growing evidence that the generation of free radicals or reactive oxygen species (ROS) might have a role in the pathogenesis of number of chronic diseases.[1] ROS are highly reactive transient chemical species with the potential to initiate cellular damage in cartilage directly and damage components of the extracellular matrix either directly or indirectly by up regulating mediators of matrix degradation.[2] These reactive molecules are formed during normal aerobic metabolism in cells causing depletion of immune system, change in gene expression and induce abnormal proteins.[3] Oxidation process is one of the most important routs for producing free radicals/ ROS in food, drugs and even living systems. It has been reported that ROS destroy antioxidant systems and exposure to oxidative stress may lead to the clinical manifestations.[1,3] Moreover, oxidative rancidity is the major cause of food quality deterioration, leading to the formation of undesirable off-flavors as well as unhealthful compounds. Antioxidants are known as molecules capable of inhibiting oxidation process in food and human system.

Nature has endowed living biological systems with antioxidants and free radical scavenger's compounds such as dietary antioxidants (ascorbic acid, β-carotene, glutathione, α-tocopherol, and uric acid), hormones (angiotensin, estrogen) and endogenous enzymes (catalase, glutathione peroxidase

Submission Date : 19-07-2014
Revision Date : 10-09-2014
Accepted Date : 23-12-2014

DOI:10.5530/ijper.49.1.8

Correspondence Address
Dr. Vivek K. Bajpai
Department of Applied Microbiology and Biotechnology, Yeungnam University, Gyeongsan, Gyeongbook 712-749, Republic of Korea.
E-mail: vbajpai04@yahoo

www.ijper.org

and superoxide dismutase).[4] These compounds are able to remove oxygen free radicals formed in cells and thus protect the human body from diseases and also retard rancidity from lipid peroxidation of foods.[4] Although application of few selected synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) has certain required level of safety parameters in food and living systems, they are known to be associated with some prompt negative health effects including malignancies, hepatic damages and toxicities in animal models.[5,6] Hence, there is an increasing trend to substitute synthetic antioxidants with naturally occurring antioxidants. Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing ROS-induced tissue injury. Moreover, well-known and traditionally used natural antioxidants of plant-based community are already exploited commercially either as antioxidant additives or a nutritional supplements.[3]

Medicinal plants are important sources of natural products which differ widely in terms of structures, biological properties and mechanisms of actions. Various phytochemical components, especially polyphenols such as tannins, flavonoids, phenyl propanoids and phenolic acids etc. are known to be responsible for antioxidant and free radical scavenging efficacy. Polyphenols possess various biological effects, which are mainly attributed to their antioxidant activities in scavenging free radicals, inhibition of lipid peroxidation and metal chelation. Generally polyphenols share the same chemical pattern; one or more phenolic groups for which they react as hydrogen donors and in that way neutralize free radicals.[7] Polyphenols are natural biologically active components found in every part of the plant including leaves, flowers, shoot, stem and root which work as defense mechanism against diseases or more accurately, to protect the plant from disease.[7]

Trachyspermum ammi is an annual herb originated in India, Iran, Egypt and Eastern region of Persia. *T. ammi* also called Ajwain is commonly used in Indian traditional system since ages as spice. In Ayurveda, *T. ammi* is used as a medicine with anti-spasmodic, stimulatory, and carminative properties. The seeds of *T. ammi* are used for food flavoring purposes as preservative.[8] *T. ammi* derived essential oil has ultimate use in perfumer industry.[8] In Indian traditional medicinal system, *T. ammi* is administered as a household remedy for stomach disorders, relieving colic pains, and a as a common remedy for asthma.[3] *T. ammi* which contains a biologically active compound thymol, has been found to possess several biological activities including anti-inflammatory, antimicrobial, antioxidant, immunomodulatory, anti-filarial, hypolipidaemic, anthelmintic, gastro-protective and nematicidal activity.[3,8-10]

However, to the best of our knowledge and literature survey, there is no any systematic report available on the antioxidant efficacy of ethanolic seed extract of *T. ammi* (ESETA). Hence, the aim of this research is to determine the potential efficacy of ethanolic seed extract of *T. ammi* (ESETA) in various antioxidant and radical scavenging models as well as lipid peroxidation inhibitory effect and phytochemical analysis.

MATERIALS AND METHODS

Chemicals and instrument

The chemicals and reagents used in this study such as DPPH, sodium nitroprusside (SNP), Griess reagent, trichloroacetic acid (TCA), bovine brain extract, nitro blue tetrazolium (NBT), ferric chloride, potassium ferricyanide, and Gallic acid, as well as standard antioxidant compounds ascorbic acid, and butylated hydroxyl anisole (BHA) were purchased from Sigma-Aldrich (St. Louis, USA) and were of analytical grade. Spectrophotometric measurements were done using a 96-well microplate ELISA reader (Tecan, Infinite M200, Mannedorf, Switzerland).

Plant material and extraction

Dried seed powder of *T. ammi* was gifted from Jeevan Herbal Product, Sagar, MP, and India. The dried seed powder (100 g) was extracted by drenching in ethanol in a conical flask for 7 days at room temperature. Further, the solvent was filtered followed by distillation under reduced pressure using a rotary evaporator (Eyela, China) until the solvent was dried completely. Finally the extract having a yield of 7.1 mg/100 g, was preserved in a sealed vial at 4°C until tested and analyzed.

Preliminary phytochemical screening

A qualitative phytochemical screening of the ethanolic seed extract of *T. ammi* (ESETA) to detect the presence of essential phytoconstituents such as alkaloid, tannin, saponin, flavonoid, anthraquinone glycoside, steroids, terpenes, glycosides, proteins, amino acids, reducing sugar and phenol was carried out using standard biochemical procedures as described.[11,12]

Determination of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity

The antioxidant activity of ESETA, based on scavenging of stable DPPH free radical was determined by our previously described method.[13] Different concentrations of ESETA (25-150 µg/ml) were added to 0.004% methanolic solution of DPPH in the ratio of 1:1 in a

96-well microplate. The mixture was incubated at 37°C in dark for 30 min with shaking (150 rpm). Absorbance was recorded at 517 nm using the Tecan ELISA reader against a blank sample. All the tests were run in triplicate. Ascorbic acid was used as reference compound in the concentration range of 25-150 µg/ml. The percent inhibition activity was calculated using the following formula:

$$\text{Percent inhibition}(\%) = \frac{\text{A control} - \text{A test}}{\text{A control}} \times 100$$

Where, A control is the absorbance of the control reaction and A test represents the absorbance of a test reaction.

Determination of nitric oxide radical scavenging activity

Sodium nitroprusside (SNP) automatically generates nitric oxide, in aqueous solution at physiological pH, which intermingles with oxygen to generate nitrite ions that can be anticipated by the Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride). Scavengers of free radicals result in the reduced production of nitric oxide radicals. In this assay, solution of SNP (10 mM) in phosphate buffer saline (PBS pH 7.4) was mixed with different concentrations of ESETA (20-100 µg/ml). The mixture was incubated at 37ºC for 60 min in light. The half quantity of aliquots was taken and mixed with equal quantity of the Griess reagent, and the mixture was incubated at 25ºC for 30 min in dark. The absorbance of pink chromophore generated during diazotization of nitrite ions with sulphanilamide and subsequent coupling with naphthyl ethylene diamine dihydrochloride (NED) was read at 546 nm against a blank as described in our previous study.[13] All the tests were performed in triplicate. Ascorbic acid was used as standard reference compounds in the concentration range of 20-100 µg/ml. The percent inhibition activity was calculated by the formula:

$$\text{Percent inhibition}(\%) = \frac{\text{A control} - \text{A test}}{\text{A control}} \times 100$$

Where A control is the absorbance of control reaction and A test is the absorbance of test reaction.

Determination of superoxide radical scavenging activity

Superoxide radical scavenging activity of ESETA was measured by the reduction of nitro blue tetrazolium (NBT) according to our previously reported method.[13] The non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system generates superoxide radicals, which reduce NBT to a purple color formazan. In this assay, the reaction mixture (150 µl) contained phosphate buffer (0.2 M, pH 7.4), NADH (73 µM), NBT (50 µM), PMS (15 µM) and various concentrations (50-250 µg/ml) of the ESETA and standard compound. After incubation for 60 min at room temperature, the absorbance of the reaction mixture was measured at 560 nm against an appropriate blank to determine the quantity of formazan generated. All tests were performed three times. Ascorbic acid was used as a standard. The percent inhibition activity was calculated by the formula:

$$\text{Percent inhibition}(\%) = \frac{\text{A control} - \text{A test}}{\text{A control}} \times 100$$

Where A control is the absorbance of control reaction and A test is the absorbance of test reaction.

Determination of hydroxyl radical scavenging activity

For determining the hydroxyl radical scavenging activity of ESETA, our previously described method was adopted.[13] The assay is based on quantification of the degradation product of 2-deoxy-2-ribose sugar by condensation with 2-thiobarbituric acid (TBA). In this assay, the hydroxyl radical was generated by the Fenton's reaction using Fe^{3+}-ascorbate-EDTA-H_2O_2 system. The reaction mixture in a total volume of 240 µl contained 2-deoxy-2-ribose (3 mM), KH_2PO_4-KOH buffer (20 mM, pH 7.4), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 (2 mM), ascorbic acid (0.1 mM) and various concentrations (100-500 µg/ml) of ESETA or standard compound. After incubation for 45 min at 37°C, 40 µl of 2.8% TCA, and 40 µl of TBA (0.5% in 0.025M NaOH solution containing 0.02% BHA) were added in the reaction mixture, and the mixture was incubated at 95°C for 15 min to develop the pink color. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. All tests were performed three times. Butylated hydroxyl anisole (BHA) was used as a reference standard. The percent inhibition activity was calculated by the formula:

$$\text{Percent inhibition}(\%) = \frac{\text{A control} - \text{A test}}{\text{A control}} \times 100$$

Where A control is the absorbance of control reaction and A test is the absorbance of test reaction.

Determination of anti-lipid peroxidation activity

Our previously reported method was adopted to determine the Fe^{3+}/ascorbic acid-dependent non-enzymatic lipid peroxidation activity of ESETA in bovine brain extract.[13] The reaction mixture, in the absence and presence of ESETA (50-250 µg/mL) or reference compound, containing 50 µl of bovine brain phospholipids (5 mg/ml), 1 mM $FeCl_3$ and 1 mM ascorbic acid in 20 mM phosphate buffer with a final volume of 330 µl, was incubated at 37°C for 1 h. The hydroxyl radicals generated in the reaction initiated the lipid

$$\text{Percent inhibition}(\%) = \frac{\text{A control} - \text{A test}}{\text{A control}} \times 100$$

peroxidation, resulting in malondialdehyde (MDA) production that was measured by thiobarbituric acid (TBA) reaction. All tests in this assay were performed three times. BHA was used as reference compound. The percent inhibition activity was calculated by the formula:

Where A control is the absorbance of control reaction and A test is the absorbance of test reaction.

Determination of reducing power activity

The ferric ion (Fe^{3+}) reducing power of the ESETA was determined by our previously described method.[13] Aliquots (50 µl) of different concentrations of ESETA (5-25 µg/ml) and/or positive control, ascorbic acid were mixed with 50 µl phosphate buffer (0.2 M, pH 6.6) and 50 µl potassium ferricyanide (1% w/v in H_2O), followed by incubation at 50°C for 20 min in dark. After incubation, 50 µl of TCA (10% w/v in H_2O) was added to terminate the reaction and the mixture was subjected to centrifugation at 3000 rpm for 10 min. For final reaction mixture, the supernatant (50 µL) was mixed with 50 µl distilled water and 10 µl $FeCl_3$ solution (0.1% w/v in H_2O). The reaction mixture was incubated for 10 min at room temperature and the absorbance was measured at 700 nm against an appropriate blank solution. A higher absorbance of the reaction mixture indicated greater reducing power ability. All tests were run in triplicate. Ascorbic acid was used as a standard drug.

Statistical analysis

All data were expressed as the mean ± SD. Analysis of variance using one-way ANOVA was performed to test the significance of differences between means at the 5% level of significance using the statistical analysis software, SAS (SAS 9.1 Version, NC, USA).

RESULTS AND DISCUSSION

Phytochemical analysis

As shown in Table 1, the ESETA was also found to possess various phytochemicals or polyphenols such as flavonoids, tannins, phenolics, steroids, glycosides, terpenoids and alkaloids, which are known to be responsible for their potent antioxidant or free radical scavenging activities. In general, the antioxidant compounds of essential oils are terpenoids, which are phenolic in nature, and it would seem rational that their antioxidant mode of action might be related to that of other compounds. Although several different methods have been developed in order to evaluate the antioxidant activity of biological samples, it is relatively difficult to measure each antioxidant component separately. Hence, in this study, we have attempted to explore new therapeutic agent of plant origin as an ESETA, and confirmed its biological efficacy in various *in vitro* antioxidant models including it potent efficacy on the inhibition of lipid peroxidation. In recent years, several researchers have reported that

Table 1: Phytochemical analysis of ethanolic seed extract of *Trachyspermum ammi*.

Tests	Ethanolic seed extract of *Trachyspermum ammi*
Alkaloids	
a). Dragendroff's test	+
b). Mayer's test	+
c). Wagner's test	+
d). Hager's test	+
Steroids / Terpenes	
a). Salkowski test	+
b). Liebermann test	+
c. Liebermann-Burchard test	+
Saponins	
a). Foam test	-
b). Haemolysis test	-
Cardiac glycosides	
a). Keller Kilani test	+
b). Legal's test	+
c). Baljet test	+
Phenols	
a). 10% ferric chloride test	+
Anthraquinone glycoside	
a). Modified Borntrager's test	-
Tannins	
a). Ferric Chloride test	+
b). Lead-acetate test	+
c.) Potassium dichromate test	+
Flavonoids	
a). Lead acetate test	+
Proteins	
a). Biuret test	-
b). Xanthoproteic test	-
c). Millon's test	-
Amino acids	
a). Ninhydrin test	-
Reducing Sugars	
a). Molisch's test	-
b). Barfoed's test	-
c). Felhing 's test	-

Note: (+) = Present and (-) = Absent

phytochemicals including alkaloids, glycosides, terpenoids, saponin, phenols and steroids have enormous antioxidant and free radical scavenging activities.[7,14] Previously it has been reported that plant extracts rich in polyphenols and essential phytoconstituents have been shown to exert potent antioxidant and free radical scavenging activities in various antioxidant models.[7,14]

DPPH radical scavenging activity

Figure 1. demonstrates a significant decrease in the concentration of DPPH radicals due to the scavenging ability of ESETA and standard compound, ascorbic acid. The scavenging effect of ESETA and standard compound at the concentration of 100 µg/ml, on the DPPH radical was found to be as 62.23%, and 72.91%, respectively. However, 150 µg/ml concentration of ESETA inhibited 73.41% of DPPH radical. The results were concentration-dependent and statistically significant ($p < 0.05$). DPPH radical scavenging activities of various plant products or extracts have also been observed previously by.[7,13,14]

A stable free radical, DPPH has been widely used as sensitive and rapid tool to estimate free radical scavenging activity of both hydrophilic and lipophilic antioxidants.[15] Antioxidants can neutralize free radicals on interaction with DPPH either by transferring electrons or hydrogen atoms to DPPH.[15] This method possibly determines the anti-radical power of an antioxidant by measuring the decrease in the absorbance of DPPH radical, resulting in color change from purple to yellow, by an antioxidant through donation of hydrogen to form a stable DPPH molecule.[16] DPPH had strong absorbance in the radical form at the wavelength of 517 nm which disappears after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule.[16]

Nitric oxide radical scavenging activity

Figure 2, shows nitric oxide radical scavenging effect of ESETA and standard compound at the concentration of 80 µg/ml which was found to be 56.42%, and 63.57%, respectively, whereas the ESETA at 100 µg/ml concentration inhibited 67.33% of nitric oxide radicals. The results were statistically significant ($p < 0.05$) and concentration dependent. The nitric oxide radical scavenging activities of various plant-based products and or extracts have been reported previously.[7,13,14] Nitric oxide, a cell signaling molecule, has been associated with variety of physiological processes in the human body which transmits signals from vascular endothelial cells to vascular smooth muscle cells causing vasodilatation.[17] It plays an essential role in vital physiological functions in respiratory, immune, neuromuscular and other systems. This molecule also regulates the release of neurotransmitter, neuronal excitability, learning and memory processes as well as inflammatory bowel syndrome, sepsis, dementia, multiple sclerosis and stroke.[17] In addition, it has been also confirmed that nitric oxide modulates neurotoxin-induced cellular damage and is involved in neuronal cell death in Parkinson's disease and other neurodegenerative disorders such as Alzheimer disease.[17] Several reports have confirmed that nitric oxide may modulate iron catalyzed oxidation reactions such as the superoxide anion driven Fenton's reaction, which produces powerful oxidants such as the hydroxyl radical and organometallic complexes.[18] The mechanisms by which nitric oxide may inhibit lipid peroxidation are not clearly defined, however, one possible mechanism relates to the ability of this molecule is to terminate propagation of lipid peroxidation reactions.

Superoxide radical scavenging activity

In this assay, superoxide anions derived from dissolved oxygen by the PMS/NADH system reduce NBT. In

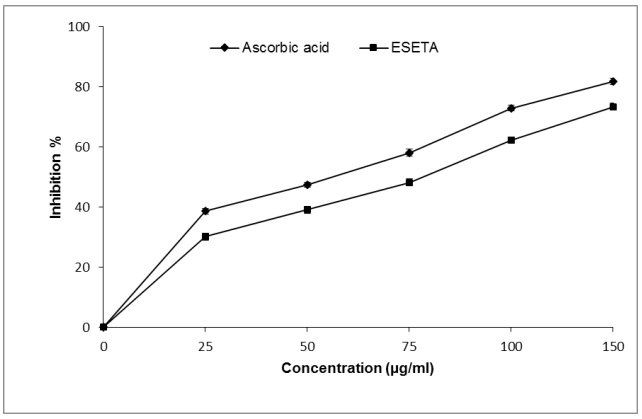

Figure 1: DPPH radical scavenging activity of ethanolic seed extract of *Trachyspermum ammi* (ESETA) and standard antioxidant compound, ascorbic acid.

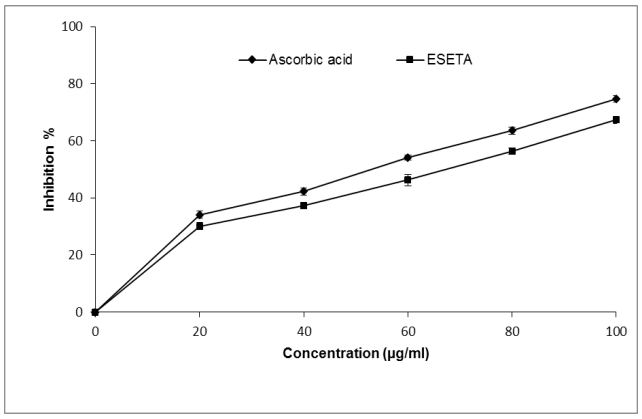

Figure 2: Nitric oxide radical scavenging activity of ethanolic seed extract of *Trachyspermum ammi* (ESETA) and standard antioxidant compound, ascorbic acid.

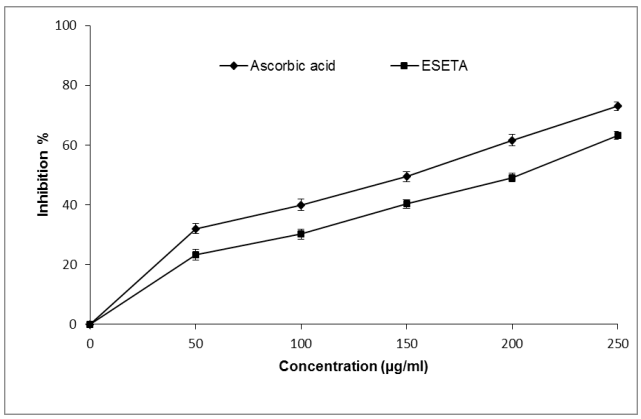

Figure 3: Superoxide radical scavenging activity of ethanolic seed extract of *Trachyspermum ammi* (ESETA), and standard compound, ascorbic acid

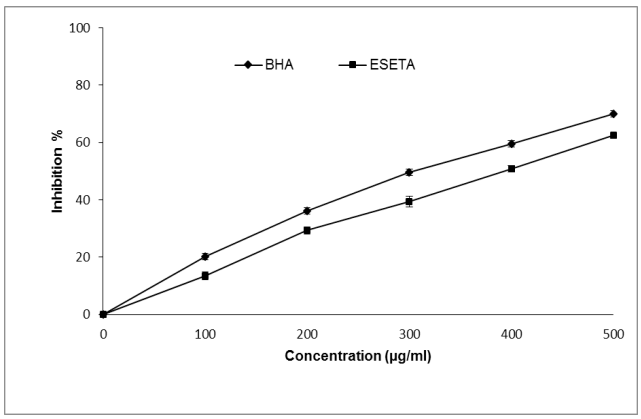

Figure 4: Hydroxyl radical scavenging activity of ethanolic seed extract of *Trachyspermum ammi* (ESETA), and standard compound, butylated hydroxyl anisole (BHA).

this method, superoxide radicals reduce the yellow dye (NBT^{2+}) to produce the blue formazan, measuring at 560 nm spectrophotometrically. Antioxidants inhibit the formation of blue colored NBT. The decrease in absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion radicals in the reaction mixture. Figure 3, shows the concentration-dependent inhibition of superoxide radical generation at all defined concentrations of ESETA and the standard compound tested. The superoxide radical inhibitory effect of ESETA, and ascorbic acid at the concentration of 250 µg/ml, was found to be 63.22%, and 73.01%, respectively. Different plant products or extracts have been found to display superoxide scavenging efficacy of great potential previously.[13,20] The human body generates highly toxic reactive oxygen species superoxide radical through different biological and metabolic reactions. Although relatively weak oxidants, superoxide exhibit only limited chemical reactivity, they are considered potential precursors of a highly reactive species such as hydrogen peroxide, hydroxyl radical and singlet oxygen, which cause lipid peroxidation.[19] Therefore, superoxide radical scavenging capacity is the first line of defense mechanism in human beings against oxidative stress. Superoxide anion is an oxygen-centered radical with a selective reactivity.[19] It has also been reported that antioxidant properties of some plant products are effective mainly via scavenging of superoxide anion radical.[18]

Hydroxyl radical scavenging activity

In this assay, the effect of ESETA on the inhibition of free radical-mediated deoxyribose damage was assessed by means of the iron (II)-dependent DNA damage assay. The Fenton's reaction generates hydroxyl radicals which degrade DNA deoxyribose sugar, using Fe^{2+} salts as an important catalytic component.[22] Oxygen radicals may attack DNA either at the sugar or the base, giving rise to a large number of products. ESETA was also capable of reducing DNA damage at all concentrations used. The ESETA was analyzed for hydroxyl radical scavenging activity to ascertain its antioxidant efficacy. As demonstrated in Figure 4, the ESETA displayed potent efficacy of hydroxyl radical scavenging activity in concentration-dependent manner. The hydroxyl radical scavenging activity of ESETA and BHA at the concentration of 500 µg/ml was found to be 62.48% and 70.02%, respectively. The ability of ESETA to quench hydroxyl radicals seems to be directly related to the prevention of propagation of the process of lipid peroxidation and seems to be good scavenger of ROS, thus reducing the rate of the chain reaction. The hydroxyl radical scavenging activity of various plant secondary metabolites and extracts has been reported previously.[13,20] The hydroxyl radical formed in biological system is an extremely reactive free radical which has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells.[21] The best characterized biologic damage caused by hydroxyl radical is its capacity to stimulate lipid peroxidation, which occurs when hydroxyl radical is generated close to the membranes and attacks the fatty acid side chains of the membrane phospholipids.[21] This radical can be formed from superoxide anion and hydrogen peroxide, in the presence of metal ions, such as Cu^{2+} and Fe^{2+}. The hydroxyl radical has the capacity to join nucleotides in DNA causing strand breakage which contributes to carcinogenicity, mutagenicity and cytotoxicity.[22] The hydroxyl radical scavenging capacity of any test compound is directly related to its antioxidant activity. The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins.[22]

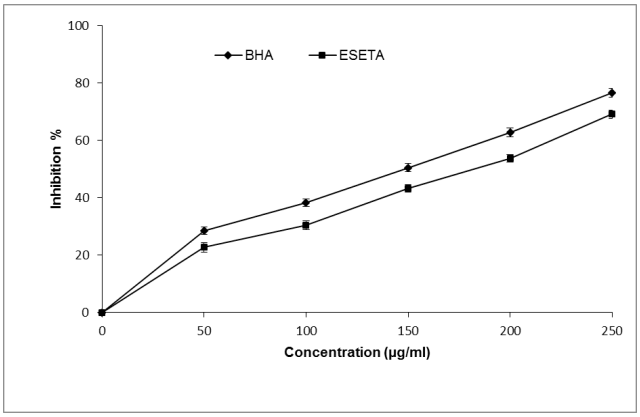

Figure 5: Lipid peroxidation inhibitory effects of ethanolic seed extract of *Trachyspermum ammi* (ESETA), and standard compound, butylated hydroxyl anisole (BHA).

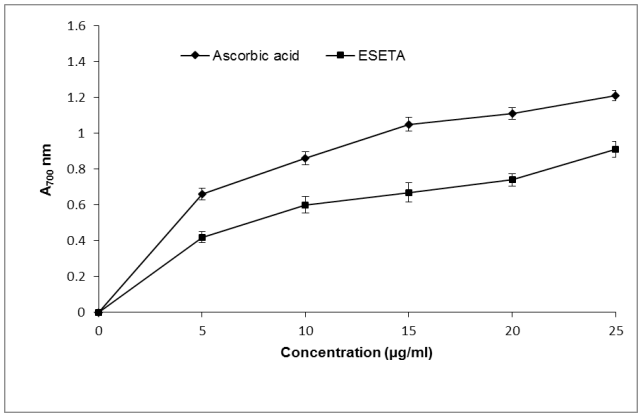

Figure 6: Reducing power activity of ethanolic seed extract of *Trachyspermum ammi* (ESETA), and standard compound, ascorbic acid.

Lipid peroxidation inhibition

This assay measured the potential of ESETA to inhibit lipid peroxidation in bovine brain extract, induced by the Fe^{3+}/ascorbate system. Figure 5, shows the inhibitory effect of the ESETA on ferric ion-induced lipid peroxidation in bovine brain homogenates. The ESETA and BHA at the concentration of 250 µg/ml showed 69.22%, and 76.59% inhibitory effect on lipid peroxides, respectively. As shown in Figure 5, the ESETA showed protective effect against ferric ion-induced lipid peroxidation considerably by reducing lipid peroxidation in a concentration-dependent manner and the results were found statistically significant. Similarly, other plant extracts have also shown protective effects against Fe^{3+}- induced lipid peroxidation[24]. The inhibition of ferric ion-induced lipid peroxidation by ESETA might be because of Fe^{3+} chelation and hydroxyl radical scavenging abilities. This capacity could be considered important since Fe^{3+} can stimulate free radical formation; thus, when complexes are formed between the ESETA and Fe^{3+}, lipid peroxidation could be prevented or reduced. The ESETA was able to scavenge hydroxyl radicals produced during Fe^{3+}-catalyzed decomposition of hydrogen peroxide in Fenton's reaction, generating the highly reactive hydroxyl radical, which initiates a process of membrane lipid peroxidation that could lead to alterations in cell structure and function.[8] Similarly we reported anti-lipid peroxidation effect of essential oil derived from *C. obtuse*.[13]

Lipid peroxidation if often considered a major cause of food deterioration which directly affects color, flavor, texture and nutritional value of food or food products.[23] During this process, free radicals take electrons from the lipids in cell membranes resulting in a loss of membrane fluidity, as well as an increase of membrane permeability and decrease in physiological performance leading to endanger cell viability.[23] The chemical structure of iron, and its capacity to drive one electron reactions, makes iron a key factor in the formation of free radicals. In biological systems, lipid peroxidation generates several aldehydes products, among which MDA is considered to be the most important derivative.[8] The process of lipid peroxidation begins by a free radical chain reaction mechanism which mostly affects polyunsaturated fatty acids, and is a major cause of cell membrane disruption and cell damage.[8] This process is initiated by hydroxyl and superoxide radicals leading to the formation of peroxy radicals that eventually propagate the chain reaction in lipids. Thus, antioxidants competent of scavenging peroxy radicals could prevent lipid peroxidation.

Reducing power ability

Figure 6, shows the reducing power capacity of the ESETA as a function of its concentrations. In this assay, it was found that the reducing power of ESETA, and ascorbic acid increased with the increase of their concentrations. The reducing power efficacy of ESETA, and ascorbic acid at the highest concentration of 25 µg/ml, was found to be 0.91 and 1.21, respectively, and the results were statistically significant ($p < 0.05$). Previously various plant extracts containing different phytochemicals have been found to display antioxidant activity through their reductive capacity in a Fe^{3+}-Fe^{2+} system.[20] The results obtained in this study indicate that the marked reducing power of ESETA seems to be attributed to the antioxidant activity of ESETA. The antioxidant activity of plants is mainly contributed by the active compounds or essential phytoconstituents present.[26] Although the activity of synthetic antioxidants is often reported to be higher than that of natural antioxidants, higher toxicity rate and carcinogenicity has prohibited their use at certain levels.[25] At certain concentrations, various herbal products and/or extracts rich in polyphenols noticeably slowed down the formation of conjugated di-olefin. Recently there is increasing

interests of polyphenolic compounds in food industry because of their inhibitory effect on lipid peroxidation and formation of off-flavors and other objectionable compounds, thereby improving the quality and nutritional value of fresh as well as processed foods.[13] Previously we have also reported reducing power ability of *C. obtuse* derived essential oil which showed potent antioxidant oxidant ability in terms of its reducing power ability.[13]

Numerous reports have confirmed that the electron donation capacity reflects the reducing power of biologically active compounds in relationship with their antioxidant activity.[25] Antioxidants are reducing agents, and inactivation of oxidants by reductants can be described as reduction-oxidation (redox) reaction in which one reaction species is reduced at the expense of the oxidation of the other.[25] The reduction of Fe^{3+} is often used as an indicator of electron donating ability, which is an important mechanism of phenolic antioxidant action. In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of Fe^{3+}-Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be then monitored by measuring the formation of Perl's Prussian blue ferric ferrocyanide, $(Fe_4[Fe(CN)_6]_3)$, at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability.

CONCLUSION

Based on the outcomes of this research, it is concluded that ethanolic seed extract of *Trachyspermum ammi* (ESETA), which contained alkaloids, glycosides, terpenoids, saponin, phenols and steroids as essential phytochemicals, exhibited significant antioxidant and free radical scavenging activities. The ESETA also exerted potent antioxidant activity as reducing power ability. Moreover, ESETA had a potent inhibitory effect on ferric ion-induced lipid peroxidation. Antioxidant activity of herbal extracts is of considerable interest to food industry which is looking for such compounds with significant biological potential to be used as alternatives to synthetic and conventional food preservation system. Hence, these findings indicate that ESETA can be a source of natural antioxidant for using in food industry against oxidative deterioration as well as a useful therapeutic agent in the prevention of oxidative stress-related degenerative diseases. Further studies are planned to isolated individual biologically active constituents from ESETA in order to confirm its precise mode of action in animal models.

CONFLICTS OF INTEREST

Authors have no conflicts of interest to declare.

REFEREENCES

1. Umar S, Asif M, Sajad M, Ansari MM, Hussain U, Ahmad W, *et al*. Anti-inflammatory and antioxidant activity of *Trachyspermum ammi* seeds in collagen induced arthritis in rats. Int J Drug Dev Res. 2012; 4(1): 210-9.
2. Hitchon CA, El-Gabalawy HS. Oxidation in rheumatoid arthritis. Arthritis Res Ther. 2004; 6(6): 265-78.
3. Saxena SN, Agarwal D, Saxena R, Rathore SS. Analysis of anti-oxidant properties of ajwain (*Trachyspermum ammi* L) seed extract. Int J Seed Spic. 2012; 2(1): 50-5.
4. Ishino K, Wakita C, Shibata T, Toyokuni S, Machida S, Matsuda S, *et al*. Lipid peroxidation generates body odor component trans-2-nonenal covalently bound to protein *in vivo*. J Biol Chem. 2010; 285(20): 15302-13.
5. Hwang JY, Chang HM. Antioxidative activity of roasted and defatted peanut kernels. Food Res Int. 2001; 34(7): 639-47.
6. Gandomi H, Abbaszadeh S, Jebellijavan A, Sharifzadeh A. Chemical constituents, antimicrobial and antioxidative effects of *Trachyspermum ammi* essential oil. J Food Process Pres; 2013 DOI: 10.1111/jfpp.12131.
7. Amari NO, Bouzouina M, Berkani A, Lotmani B. Phytochemical screening and antioxidant capacity of the aerial parts of *Thymelaea hirsuta* L. Asian Pac J Trop Dis. 2014; 4(2): 104-9.
8. Baratta TM, Dorman DHJ, Deans SG. Chemical composition antimicrobial and antioxidative activity of Laurel, Sage, Rosemary, Oregano, and Coriander essential oils. J Essen Oil Res. 1998; 10(6): 618-27.
9. Jeet K, Devi N, Thakur N, Tomar S, Shalta L, Thakur R. *Trachysperumu ammi* (Ajwain): A comprehensive review. Int Res J Pharm. 2012; 3(5): 133-8.
10. Paul S, Dubey RC, Maheshwari DK, Kang SC. *Trachyspermum ammi* (L.) fruit essential oil influencing on membrane permeability and surface characteristics in inhibiting food-borne pathogens. Food Control 2011; 22(5): 725-31.
11. Edeoga HO, Okwa DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Bio technol. 2005; 4(7): 685-8.
12. Patra JK, Panigrahi TK, Rath SK, Dhal NK, Thatoi HN. Phytochemical screening and antimicrobial assessment of leaf extracts of *Excoecaria agallocha* L: A mangal species of Bhitarkanika, Orissa, India. Adv Nat Appl Sci. 2009; 3(2): 241-6.
13. Farhan H, Malli F, Rammal H, Hijazi A, Bassal A, Ajouz N, Badran B. Phytochemical screening and antioxidant activity of Lebanese *Eryngium creticum* L. Asian Pac J Trop Biomed. 2012; 2(3): S1217-20.
14. Archana B, Dasgupta N, De B. *In vitro* study of antioxidant activity of *Syzygium cumini* fruit. Food Chem. 2005; 90(4): 727-33.
15. Matthaus B. Antioxidant activity of extracts obtained from residues of different oilseeds. J Agric Food Chem. 2002; 50(12): 3444-52.
16. Aliev G, Palacios HH, Lipsitt AE, Fischbach K, Lamb BT, Obrenovich ME, *et al*. Nitric oxide as an initiator of brain lesions during the development of Alzheimer disease. Neurotox Res. 2009; 16(3): 293-305.
17. Dadashpour M, Rasooli I, Sefidkon F, Rezaei MB, Alipour D, Astaneh S. Lipid peroxidation inhibition, superoxide anion and nitric oxide radical scavenging properties of *Thymus daenensis* and *Anethum graveolens* essential oils. J Med Plants. 2011; 10(37): 109-120.
18. Kanatt KR, Chander R, Sharma A. Antioxidant potential of mint (*Mentha spicata* L.) in radiation processed lamb meat. Food Chem. 2007; 100(2): 451-8.
19. Hazra B, Sarkar R, Biswas S, Mandal N. Comparative study of the antioxidant and reactive oxygen species scavenging properties in the extracts of the fruits of *Terminalia chebula*, *Terminalia belerica* and *Emblica officinalis*. BMC Complement Alt Med. 2010; 10(1): 1-15.
20. Halliwell B. Reactive oxygen species in living systems: source, biochemistry and role in human disease. Am J Med. 1991; 91(13): 14-22.
21. Valko M, Leibfritz D, Moncola J, Cronin MTD, Mazura M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007; 39(1): 44-84.
22. Balu M, Sangeetha P, Haripriya D, Panneerselvam C. Rejuvenation of antioxidant system in central nervous system of aged rats by grape seed extract. Neurosci Lett. 2005; 383(3): 295-300.
23. Geetha RK, Vasudevan DM. Inhibition of lipid peroxidation by botanical extracts of *Ocimum sanctum*: *in vivo* and *in vitro* studies. Life Sci. 2004; 76(1): 21-8.
24. Gulcin I, Elias R, Gepdiremen A, Chea A, Topal F. Antioxidant activity of bisbenzylisoquinoline alkaloids from *Stephania rotunda*: cepharanthine and fang chino line. J Enzym Inhib Med Chem. 2010; 25(1): 44-53.
25. Wojdylo A, Oszmianski J, Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem. 2007; 105(3): 940-9.