ORIGINAL ARTICLE

Evaluation of antioxidant activity of essential oil from Ajwain (*Trachyspermum ammi*) seeds

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Background: Free radicals provoke chain reactions within the cell and cause damage to the cell. Oxidation of bio molecules such as carbohydrate, protein, lipid and nucleic acid leads to the production of free radicals which induce the onset of disease. Antioxidants scavenge these free radicals, thereby protecting the cell from damage. Phenolic compounds found in varieties of seeds and fruits are thought to have antioxidant activities. **Aim:** The present study was designed to examine the *in vitro* antioxidant activity of *Trachyspermum ammi* (Ajwain) essential oil. **Materials and Methods:** The antioxidant activity of the oil was assessed by using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH), H_2O_2 radical scavenging activity and ferric reducing antioxidant power (FRAP). The values were compared with those obtained with standard antioxidant, ascorbic acid. **Results:** It was determined that the essential oil possessed a high degree of FRAP followed by a good DPPH radical scavenging activity and a moderate H_2O_2 radical scavenging activity. **Conclusion:** This study concludes that the essential oil of Ajwain could contribute as a highly significant bio resource of antioxidants to be used in our day-to-day life in food and pharmaceutical industry.

Key words: 2,2-diphenyl-1-picryl-hydrazyl, antioxidant activity, essential oil, ferric reducing antioxidant power, H_2O_2, thymol, *Trachyspermum ammi*

INTRODUCTION

Excessive production of free radicals and the unbalanced mechanisms of antioxidant protection lead to oxidative stress, which is one of the crucial causative factor in elicitation of many chronic and degenerative diseases including atherosclerosis, cancer, diabetes mellitus, Parkinson's disease, immune dysfunction and is even involved in aging.[1-3] Both exogenous and endogenous antioxidants, either synthetically prepared or naturally obtained can be effective in prevention of the free radical formation by scavenging and promoting the decomposition and suppression of such disorder.[4,5] The use of synthetic antioxidants, such as butylated hydroxytoluene, butylated hydroxyanisole, tert-butylhydroquinone in food is admonished due to their potential as perceived carcinogens.[6] As a result, there has been a growing interest in natural resources of antioxidants that are supplied to human and animals as diet or as specific pharmaceutics[7,8] to replace synthetic antioxidants, which are being restricted due to their carcinogenicity.[9]

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Current use of plant based natural antioxidants in the form of phenolic compounds such as flavonoids, phenolic acids and tocopherols in food as well as in medicinal world are alluring much recognition because of their comparatively safe status and their putative protective effects against the deleterious oxidation-induced injuries.[10,11]

Epidemiological and *in vitro* studies on medicinal plants and vegetables have strongly supported the phenolic compounds, which are widely distributed in many fruits, vegetables and tea is believed to account mainly for the antioxidant capacity of many plants.[12]

One such plant *Trachyspermum ammi,* commonly known as Ajwain belongs to family *Apiaceae.* It is distributed throughout India and it is mostly grown in Gujarat and Rajasthan. Based on its traditional use[13] since a long period of time for treatment of inflammatory diseases and disorders of the digestive tract, it is often assumed to be safe. It has the advantage over various other plant products, which show antioxidant activity because of its easy availability and cost effectiveness.

Seeds of Ajwain are reported to possess antimicrobial,[14] hypolipidemic,[15] antihypertensive,[16] antispasmodic,[16] antilithiasis and diuretic.[17] Antitussive,[18] nematicidal,[19] antihelminthic,[20] antifilarial[21] activities. In the essential oil of Ajwain (E.O.A), the principle active constituents are phenols, mainly thymol (35-60%),[22] which majorly contributes to its curative properties.

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Hence, the present study was carried out to determine the antioxidant effects of E.O.A seeds at different concentrations.

MATERIALS AND METHODS

Chemicals and Reagents

Chemicals

2,2-diphenyl-1-picryl-hydrazyl (DPPH), H_2O_2, 2,4,6-tripyridyl-*s*-triazine (TPTZ) and ascorbic acid were obtained from Himedia. All other reagents used were of analytical grade.

Collection of plant material

Ajwain seeds were purchased from local market and identified from University of Rajasthan, Jaipur, Rajasthan.

Extraction of essential oil

The E.O.A was extracted by hydro-distillation using Clevenger's apparatus. The plant material (50 g) was crushed to powder and placed into flasks (1 L) with sterile water (250 ml) at 70°C. After 3 h the oil was isolated, dried over anhydrous sodium sulphate[23] and kept in screw capped bottles at 4°C for further use.

The oil was dissolved in dimethyl sulfoxide at different concentration (1.25-10 µl/ml) for the experimental procedure.

Chemical Characterization

Thin layer chromatography

The fraction of essential oil was further purified by TLC. TLC on analytical silica gel plates (20 cm × 20 cm Merck) with benzene: Chloroform (3:1 v/v) as mobile phase was carried out. These TLC plates were then analysed under ultraviolet (UV) lamp (366 nm). The qualitative evaluation of the plate was carried out by determining the migrating behaviour of the separated substances given in the form of retention factor (R_f value).[24] The standard R_f value of thymol is 0.64.

Evaluation of Antioxidant Potential

The extracts were evaluated for antioxidant potential through *in vitro* model systems such as DPPH, H_2O_2 radical scavenging activity and iron reducing capacity (reducing power).

DPPH radical scavenging activity

To 1 ml of DPPH dissolved in methanol (0.33%), 1 ml of (1.25-10 µl/ml) essential oil/ascorbic acid was added. After the incubation for 30 min, at 37°C, the absorbance at 517 nm was measured using UV-spectrophotometer. Corresponding blanks were taken for the same. The experiment was performed in triplicate. The absorbance of DPPH as control was obtained at 518 nm. Lower absorbance of the reaction mixture was an indication of higher radical scavenging activity of essential oil/standard antioxidant. DPPH become a stable diamagnetic molecule by accepting an electron. The methanolic solution of DPPH (violet colour) has got a strong UV absorbance at 517 nm. The presence of a reducing environment in the solution pairs the odd electrons of DPPH radical and the solution in turn losses its colour stoichiometrically and also decreases the absorbance at 517 nm.[25] The DPPH scavenging activity (%) was measured using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100 \quad (1)$$

Where,

$Abs_{control}$ is the absorbance of DPPH radical + methanol

Abs_{sample} is the absorbance of DPPH radical + essential oil/standard

Hydrogen peroxide radical scavenging activity

A total of 4 ml of (1.25-10 µl/ml) essential oil/ascorbic acid was added to 0.6 ml of hydrogen peroxide solution (4 mM) in phosphate buffer (0.1 M and pH-7.4). After incubating for 10 min at 37°C, the absorbance at 230 nm was measured. Corresponding blanks were taken. The experiment was performed in triplicate. The absorbance of phosphate buffer as control was measured at 230 nm.[26] Hydrogen peroxide produces hydroxyl radicals in cells. Scavenging of these radicals is seen by the decrease in absorbance at 230 nm with increasing concentration of the test sample. The scavenging effect (%) was measured using equation (1).

Ferric reducing antioxidant power assay

The antioxidant activity was also measured by applying FRAP assay of Benzie and Strain, 1999.[27] FRAP assay uses antioxidant as reductants in a redox linked calorimetric method, which employs an easily reduced oxidant system present in stoichiometric excess. At low pH, reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form (which has an intense blue colour) was checked by measuring the change in absorption at 593 nm. The change in absorbance seen is directly related to the combined or "total" reducing power of the electron donating antioxidants present in the reaction mixture.

A total of 100 µl of essential oil was mixed with 3 ml of working FRAP reagent and absorbance at 593 nm was measured at 0 min after vortexing. Thereafter, samples were placed at 37°C in water bath and absorbance was again measured after 4 min. Ascorbic acid standards were processed in the same way.

The FRAP value was determined by using the following equation:

FRAP value of Sample (µM) = (Change in absorbance of sample from 0 min to 4 min/change in absorbance of standard from 0 min to 4 min) × FRAP value of standard at different concentrations.

Note: FRAP value of ascorbic acid is 2.[27]

RESULT AND DISCUSSION

Yield of Ajwain seed essential oil is given by the following formula and the % yield of Ajwain was found to be 1.2%.

$$\%\,\text{yeild (w/v)} = \frac{\text{weight of the extract}}{\text{weight of ground plant part}} \times 100$$

TLC

TLC was performed to confirm the presence of thymol, the probable reason why Ajwain shows inhibitory effects towards various diseases. The result showed the spot with R_f value = 0.68. It was concluded that this fraction of essential oil was thymol (R_f value = 0.64.).

Antioxidant Activity

DPPH radical scavenging activity

The evaluation of the Antioxidant power by DPPH radical scavenging activity has been widely in use for different plant extracts and foods.[28] DPPH is a stable free radical, which changes its colour from violet to yellow upon reduction by the process of electron donation. E.O.A when reacts with it, convert it to 1,1-diphenyl-2-(2,4,6-trinitophenyl) hydrazine. The scavenging potential of the antioxidants present in E.O.A can thus be determined by their degree of discoloration to yellow.

As per the data obtained through experiment, it was seen that the scavenging effect of DPPH radical increased with increasing the concentration of essential oil (1.25-10 µl). The E.O.A has shown considerable reducing power. They exhibited moderate scavenging activity when compared with ascorbic acid (standards).

Concentration of the sample necessary to decrease initial concentration of DPPH by 50% (IC_{50}) under the experimental condition was calculated, which indicated that the E.O.A seeds displayed the highest DPPH scavenging effect at concentration 2.5 µl/ml.

In this study, the ability of samples to scavenge DPPH radical was determined on the basis of their concentration providing % scavenging effect. Table 1 and Figure 1 shows the dose-response curve of DPPH radical scavenging activity of E.O.A compared with ascorbic acid.

H_2O_2 radical scavenging activity

Scavenging of OH - is an important antioxidant activity because of its very high reactivity, which can easily cross the cell membranes at specific sites, react with most biomolecules and furthermore cause tissue damage and cell death. Thus, removing OH - is very important for the protection of living systems.[29] Table 2 and Figure 2 shows the OH − scavenging effect of E.O.A at different dose levels. The H_2O_2 radical scavenging ability of the essential oil was higher than ascorbic acid at same concentration and it was best shown at 10 µl/ml Concentration (39.32%),

Table 1: DPPH assay for antioxidant activity of E.O.A

Concentration (µl/ml)	% scavenging activity of E.O.A.	% scavenging activity of ascorbic acid
1.25	32.89±1.56	96.11
2.5	42.88±0.78	96.67
5.0	67.28±2.35	96.67
10.0	76.43±1.18	97.22

E.O.A – Essential oil of Ajwain; DPPH – 2-diphenyl-1-picryl-hydrazyl

Table 2: H_2O_2 assay for antioxidant activity of E.O.A

Concentration (µl/ml)	% scavenging activity of E.O.A	% scavenging effect of ascorbic acid
1.25	4.62±1.30	8.11
2.5	16.17±0.60	13.21
5.0	27.31±0.52	24.14
10.0	39.23±0.76	31.43

E.O.A – Essential oil of Ajwain

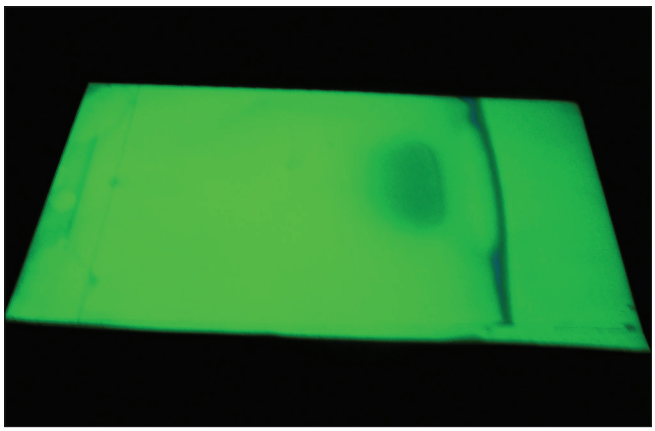

Figure 1: Thin layer chromatography of essential oil of Ajwain

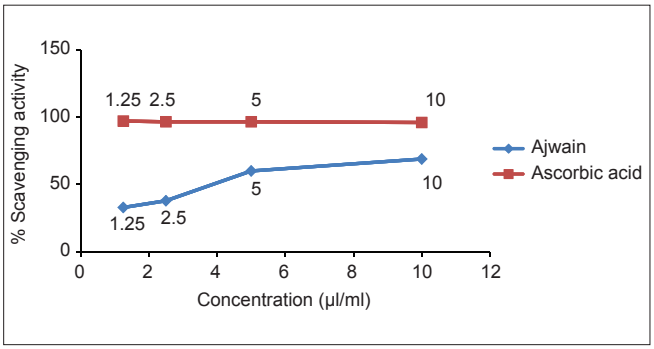

Figure 2: 2,2-diphenyl-1-picryl-hydrazyl radical scavenging activity of essential oil of Ajwain at different concentrations

the same concentration at which ascorbic acid gave 31.43%. The ability of E.O.A to quench hydroxyl radical seems directly related to the process of preventing lipid peroxidation thus proving to be good scavengers of active oxygen species.

Scavenging of H_2O_2 by essential oil may be attributed to its phenolics constituents (thymol), which can donate electrons to H_2O_2 radical, thus neutralizing it to water.[30,31]

Reducing ability (FRAP) assay

FRAP assay is a direct way of measuring antioxidants or reductants in a sample that react with oxidized ferric tripyridyl triazine (Fe^{3+} TPTZ) complex and produces reduced and coloured ferrous tripyridyl triazine (Fe^{2+} TPTZ). The antioxidant activity of E.O.A showed higher FRAP value with increasing concentration, which was higher than that of ascorbic acid (showed constant results with increasing concentration). Table 3 and

Table 3: FRAP assay for antioxidant activity of E.O.A

Concentration (µl/ml)	FRAP value of E.O.A	FRAP value of ascorbic acid
1.25	30.00±2.00	2
2.5	57.33±1.15	2
5.0	102.00±0.00	2
10.0	196.67±5.03	2

Values are expressed as mean±standard deviation of observations. E.O.A – Essential oil of Ajwain; FRAP – Ferric reducing antioxidant power

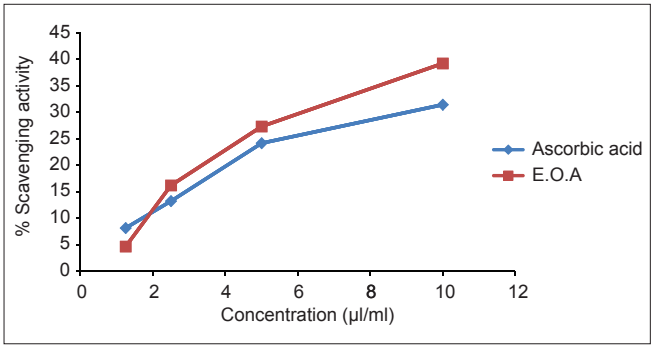

Figure 3: % Scavenging activity of essential oil of Ajwain at different concentrations against H_2O_2

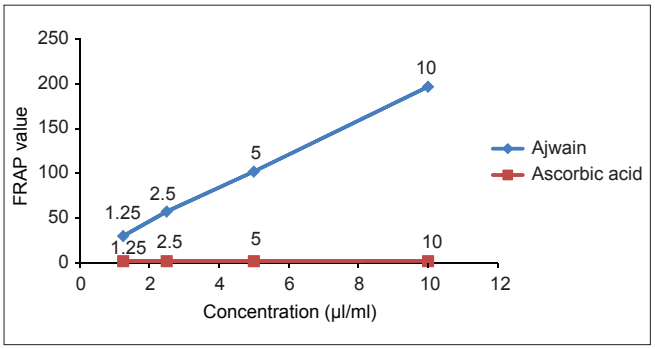

Figure 4: Ferric reducing antioxidant power value of essential oil of Ajwain at different concentrations

Figure 3 shows the dose-response curve of reducing ability of E.O.A. Rice-Evans[32] reported that phenolic compounds acts as reducing agents, hydrogen donators and singlet oxygen quenchers. According to recent reports, a highly positive relationship between total phenols and antioxidants activity appears to be the trend in many plant species.[33] Thus, the essential oil might have reacted with the free radicals, donated electrons to them and thus terminated the chain reaction [Figure 4].

CONCLUSION

The studies indicated that there is a close relationship between diet, life-style and human diseases. The proposed outcome of the study is selection and formulation of optimum dose and to develop poly-herbal drug for therapeutic purpose. The study can be further extended to evaluate the efficacy *in vivo*.

Additional *in vivo* studies and clinical trials would be needed to justify and further evaluate the potential of the E.O.A as antioxidant agent in topical or oral applications.

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