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ORIGINAL ARTICLE

Estimation of Phenolic Components and *in vitro* Antioxidant Activity of Fennel (*Foeniculum vulgare*) and Ajwain (*Trachyspermum ammi*) seeds

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ABSTRACT

Antioxidants are substances that protect cells from the cellular damage caused by unstable molecules known as free radicals induced oxidative stress. Antioxidants neutralize free radicals as a natural by-product of normal cell processes. Phenolic compounds, which are widely distributed in many fruits, vegetables, and tea are believed to account mainly for the antioxidant capacity of many plants. In the present study methanolic and aqueous seed extracts of fennel (Foeniculum Vulgare) and ajwain (Trachyspermum ammi) were studied for the quantification of the total antioxidant capacity and determination of total phenolic compounds. The total antioxidant capacity was measured using DPPH, H_2O_2 and FRAP methods and the total phenolic compounds were measured using the Folin- Ciocalteu reagent in an effort to validate the medicinal potential of the herb. Ascorbic acid was used as standard.

The antioxidant activity expressed as IC_{50} displayed the similar DPPH• scavenging effect in methanol and aqueous seed extract of fennel and ajwain, less than ascorbic acid at 30 g/ml. The methanolic extract of fennel seeds showed the highest OH- scavenging potential of 71.61% at 240 g/ml concentration. The reducing ability (FRAP activity) of the extracts were in the range of 7 – 48 m Fe (II)/g. Polyphenolic compounds are known to have antioxidant activity and it is likely that the antioxidant activity of the extracts is due to these compounds. The level of the phenolic compounds in the methanolic and aqueous seed extracts of fennel and ajwain were considerable. The study showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

Keywords*: Antioxidant, Polyphenolic compounds, Oxidative stress, Free radicals*

INTRODUCTION

Generally, the amount of reactive oxygen species (ROS) and antioxidants produced in the body are balanced, but somehow under some unavoidable circumstances, antioxidant defense mechanism proves to be insufficient to compensate the highly enhanced ROS which is harmful for the body [1]. Likewise, various toxicants act through the ROS like superoxide anions (O_2^-), hydroxyl radicals (HO.) and non free radical species such as H_2O_2, singled oxygen (O_2) and nitric oxide (NO) that play a major role in initiation of degenerative processes such as cellular damage that may be related to many body ailments viz. heart diseases, cancer, aging [2].

Antioxidants are intimately involved in prevention of cellular damage. Catalase, superoxide dismutase and glutathione peroxidase are some of the natural antioxidants found in body. They neutralize free radicals as the natural by-product of normal cell processes [3]. Since ancient time, in vitro studies on medicinal plants and vegetables have shown growing interest towards natural antioxidants from herbal sources in the form of phytochemicals which exert a protective effect against oxidative stress in biological systems [4]. Phenolic compounds with antioxidant activity, which are widely distributed in many fruits, vegetables, and tea are believed to account mainly for antioxidant capacity of many plants [5].

Fennel (*Foeniculum vulgare* Mill.) and ajwain (*Trachyspermum ammi*) both are well known Umbeliferous plants from Apiaceae family. Fennel is chiefly known as culinary herb but it is a commonly used household remedy for various medicinal purposes such as anticancer [6], anti-inflammatory [7] and antioxidant [8] agent. Ajwain (*Trachyspermum ammi*) is a popular spice and traditionally used in Indian system of medicine to treat amoebiasis, febrile conditions, stomach disorders, dyspepsia and disorders of inflammation.

In context to the present study , antioxidant activity of two commonly known spices fennel (*Foeniculum vulgare* Mill.) and ajwain (*Trachyspermum ammi*) seeds were evaluated. Besides, phytochemical screening and estimation of total phenolic content of the same plant material was done to check their contribution in antioxidant activity.

MATERIALS AND METHODS

Plant seed collection and identification

Seeds of fennel and ajwain were purchased from local market and identified from Agricultural university , Jobner ,Rajasthan .

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.

Preparation of extract

Samples were extracted according to Wattasinghe et al 2002 . with slight modifications [9] . 6 g of powdered seeds were extracted with 100 ml methanol (90% v/v) for methanolic extract (with distilled water for aqueous extract) in an glass container for 7-8 days. The resulting slurry was oven dried , the residue was twice re-extracted with methanol under the same conditions and filtered using Whatman No. 1 filter paper and 0.45 m Millipore nylon membrane filters.

Different concentrations like 30µgm, 60µgm, 120µgm and 240 µgm/ml of AMSE (Ajwain methanolic seed extract) and FMSE (Fennel methanolic seed extract) or AASE (Ajwain aqueous seed extract) and FASE (Fennel aqueous seed extract) were made by serial dilution. The extract was stored at 4°C for future usage.

Phytochemical Analysis

Phytochemical analysis was carried out using biochemical tests as proposed by Indian Pharmacopia and Harborne Vargas [10 , 11] . By these methods , the presence of several phytochemicals like alkaloids (Dragendroff's test [12]), flavonoids (FeCl3 test [13]) tannins (Lead acetate test [13]), saponins (Frothing test [12]) and steroids (Libarman - Burchard's test [12]) were tested.

Estimation of total phenolic compounds

Total phenol

The total phenolic content in the aqueous and methanolic seed extract of ajwain and fennel was determined spectrophotometrically with Folin-Ciocalteau reagent [14] . An aliquot of the extract (0.5 ml) was mixed with 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (75% w/v). The resulting mixture was vortexed for 15 s and incubated at 40°C for 30 min for colour development. The absorbance of the samples was measured at 765 nm using UV/visible spectrophotometer. Total phenolic content was expressed as mg/g tannic acid equivalent from the calibration curve using the equation:

$$Y = 0.1216x, R^2 = 0.936512$$

Where; x was the absorbance and Y was the tannic acid equivalent (mg/g).

Total flavonoids

The estimation of total flavonoid content of the extract solution was based on the formation of a complex flavonoid – aluminium [14,15] . A volume of 0.5 ml of 2 % $AlCl_3$ ethanol solution was added to 0.5 ml of extract solution. After one hour of Incubation at the room temperature, the absorbance was measured at 420 nm using UV-VIS spectrophotometer. All values were calculated using the equation:

$$Y = 0.0255x, R^2 = 0.9812$$

where x was the absorbance and Y the quercetin equivalent (mg/g)

Total flavonols

Total flavonol content was determined by adopting the procedure described by Kumaran and Karunakaran [16] . The reacting mixture consisted of 2.0 ml of the sample, 2.0 ml of $AlCl_3$ prepared in ethanol and 3.0 ml of (50 g/l) sodium acetate solution. The absorption at 440 nm was read after 2.5 h at 20°C [14]. Total flavonoid content was calculated as quercetin (mg/g) equivalent from the calibration curve using the equation:

$$Y= 0.0255x, R^2 = 0.9812$$

Where; x was the absorbance and Y the quercetin equivalent (mg/g).

Total proanthocyanidins

To 0.5 ml of extract solution was added 3 ml of vanillin – methanol (4 % v/v) and 1.5 ml of hydrochloric acid and then vortexed. The absorbance of resulting mixture was measured at 500 nm after 15 min at room temperature [14,17]. Total proanthocyanidin content was expressed as catechin equivalents (mg/g) using the following equation from the calibration curve:

$$Y = 0.5825x, R^2 = 0.9277$$

Where; x was the absorbance and Y the catechin equivalent (mg/g).

***In vitro* Antioxidant Methods**

DPPH (1, 1diphenyl2picryl hydrazyl) radical scavenging activity

1 ml of (30-240 g/ml) seed extract/standard (Ascorbic acid) was added to 1 ml of DPPH in methanol (0.33%). After keeping for 30 minutes at 37° C the absorbance at 518nm was measured using UV - spectrophotometer. Corresponding blanks were taken. The absorbance of DPPH as control was measured at 518 nm. Lower absorbance of the reaction mixture indicated higher radical scavenging activity . DPPH accepts an electron to become a stable diamagnetic molecule . The methanolic solution of DPPH (violet color) has got a strong UV absorbance at 518 nm. The presence of a reducing agent in this methanolic solution pairs the odd electrons of DPPH radical and further the solution losses color stoichiometrically and also the absorbance of the solution decreases at 518 nm [18].

The scavenging effect (%) was measured using the following formula :

$$\text{Scavanging Effect(\%)} = \frac{\text{(Control absorbance – Test absorbance)}}{\text{Control Absorbance}} \times 100$$

Hydrogen peroxide radical scavenging activity Aldolfo et al. (2002)

3.4 ml of (20- 200 g/ml) seed extract/standard (Ascorbic acid) was added to 0.6ml of hydrogen peroxide solution (43mM) in phosphate buffer (PH-7.4). After incubating for 10 minutes at 37°C the absorbance was measured at 230nm. Corresponding blanks were taken. The absorbance of phosphate buffer as control was measured at 230 nm [19] . Hydrogen peroxide produces hydroxyl radicals in cells. Scavenging of these radicals by the seed extract is used as a test for antioxidant activity. The reduction of these radicals is seen by the decreased absorbance at 230 nm with increasing concentration of the test sample.

Ferric reducing / antioxidant power (FRAP) assay

Total antioxidant activity was also measured by ferric reducing antioxidant power (FRAP) assay [20] . FRAP assay uses antioxidants as reductants in a redox - linked colorimetric method, employing 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) can be monitored by measuring the change in absorption at 593 nm. The change in absorbance is directly related to the combined or “total” reducing power of the electron donating antioxidants present in the reaction mixture.

100 l of seed extract mixed with 3 ml of working FRAP reagent and absorbance was measured at zero minute after vortexing at 593 nm. Thereafter, samples were placed at 37°C in water bath and absorption is again measured after 4 minutes. Ascorbic acid standards were processed in the same way. OD was measured of standard and test at zero minute and again after four minutes at 593 nm.

For FRAP Assay results were calculated as follows:

FRAP value of sample (M) = (Change in absorbance of sample from 0 to 4 minute / Change in absorbance of Standard from 0 to 4 minute) X FRAP value of standard (1000 M).

FRAP value of Ascorbic acid is 2.

RESULT AND DISCUSSION

Phytochemical analysis

The phytochemical test performed showed positive results for carbohydrates, reducing sugars, secondary metabolites such as tannins , flavonoids, saponins, terpenes etc in methanolic and

aqueous extract of ajwain (table 1) and fennel (table 2). They play an important role in exhibiting the inhibitory role against the chemical mutagens and are responsible for antioxidant property.

Table 1 . Phytochemical screening of methanolic and aqueous seed extract (A.S.E.) of *Trachyspermum ammi* (Ajwain)

S.NO	Constituents	Methanol extract	Aqueous Extract
1.	**Carbohydrates** i.Molisch test ii. Barfoed's test iii. Fehling (reducing sugar test) iv. Fehling (combined reducing sugar test) (sofowara,1993)	_ ++ + + ++	++ + + ++
2.	**Alkaloid** Dragendroff's test (sofowara,1993)	+	++
3.	**Flavonoids** FeCl3 test (Trease and Evans,2002)	+	+
4.	**Saponins** Frothing test (sofowara,1993)	+	+
5.	**Terpenes and Steroids** Libarman-Burchard's test (sofowara,1993)	+	+
6.	**Tannins** i.FeCl3 test ii. Lead acetate test (Trease and Evans,2002)	+ ++	+++ ++
8.	**Anthraquinones test:** Borntragen's test	-	-

-= Negative (absent); + = Posotive(slightly present);++ = Positive (moderately present)

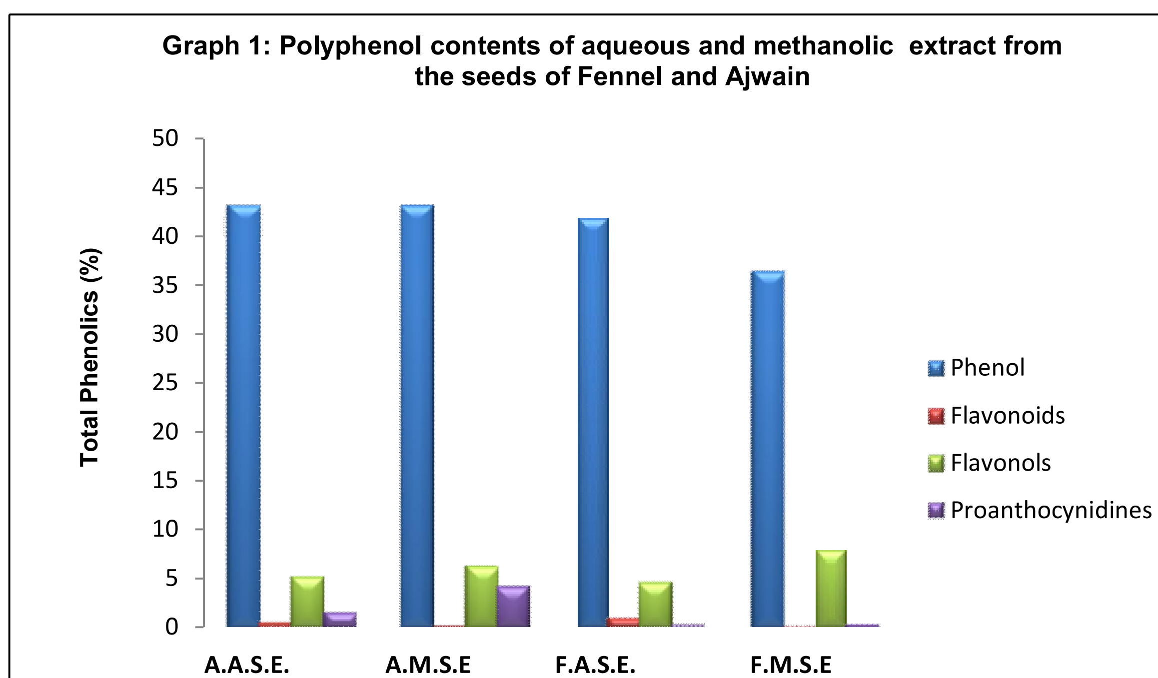
Estimation of total polyphenol contents

Results obtained in the present study revealed that the level of the phenolic compounds in the methanol extracts and aqueous extract of the seeds of *Foeniculum vulgare* and *Trachyspermam ammi* were considerably significant (Graph 1) . Polyphenolic compounds are known to have antioxidant activity and it is likely that the activity of the extracts is due to these compounds . This activity is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [21]. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases [22]. The results strongly suggest that phenolics are important components of this plant, and some of its pharmacological effects could be attributed to the presence of these valuable constituents.

Table 2. Phytochemical screening of methanolic and aqueous seed extract (F.S.E.) of *Foeniculum vulgare* (Fennel)

S.NO	Constituents	Methanol extract	Aqueous Extract
1.	**Carbohydrates** i.Molisch test ii. Barfoed's test iii. Fehling (reducing sugar test) iv. Fehling (combined reducing sugar test) (sofowara,1993)	 ++ + + +	 ++ ++ + +
2.	**Alkaloid** Dragendroff's test (sofowara,1993)	+	+
3.	**Flavonoids** FeCl3 test (Trease and Evans,2002)	+	+
4.	**Saponins** Frothing test (sofowara,1993)	+	+
5.	**Terpenes and Steroids** Libarman-Burchard's test(sofowara,1993)	+	+
6.	**Tannins** i.FeCl3 test ii. Lead acetate test (Trease and Evans,2002)	 + +	 ++
8.	**Anthraquinones:** Borntragen's test	-	-

-= Negative (absent); + = Posotive(slightly present);++ = Positive (moderately present)

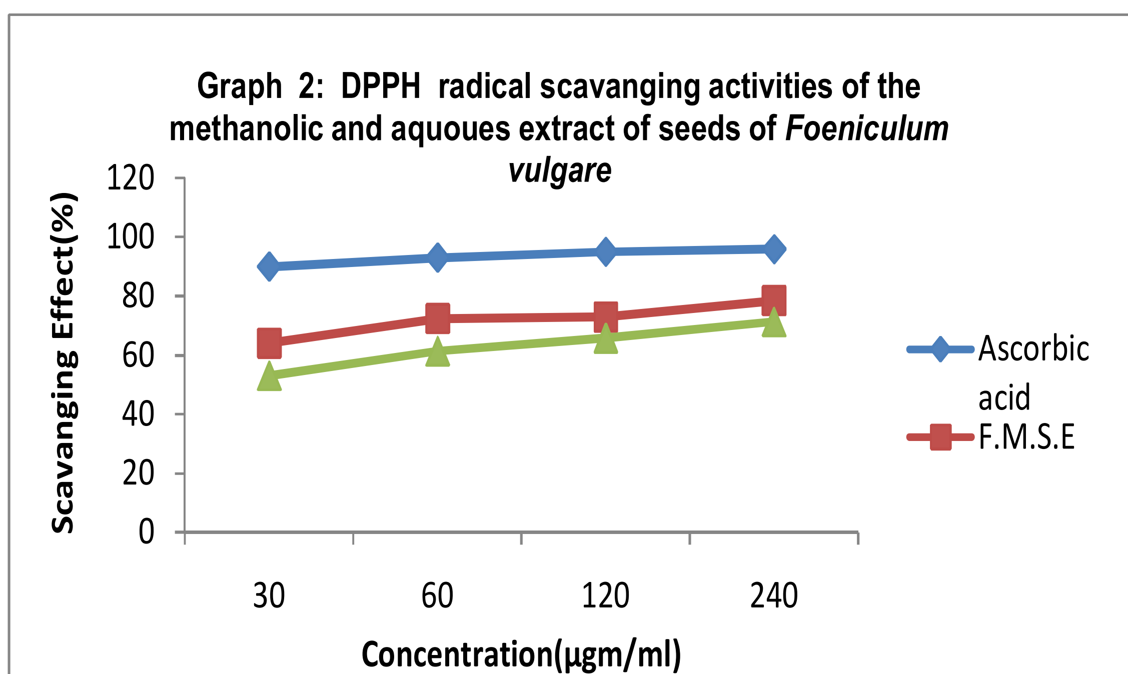


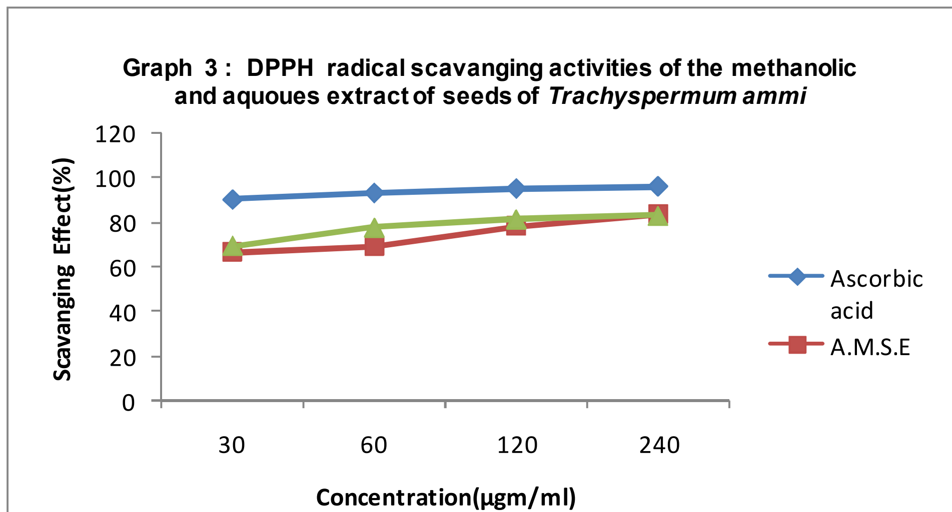
DPPH radical scavenging activity

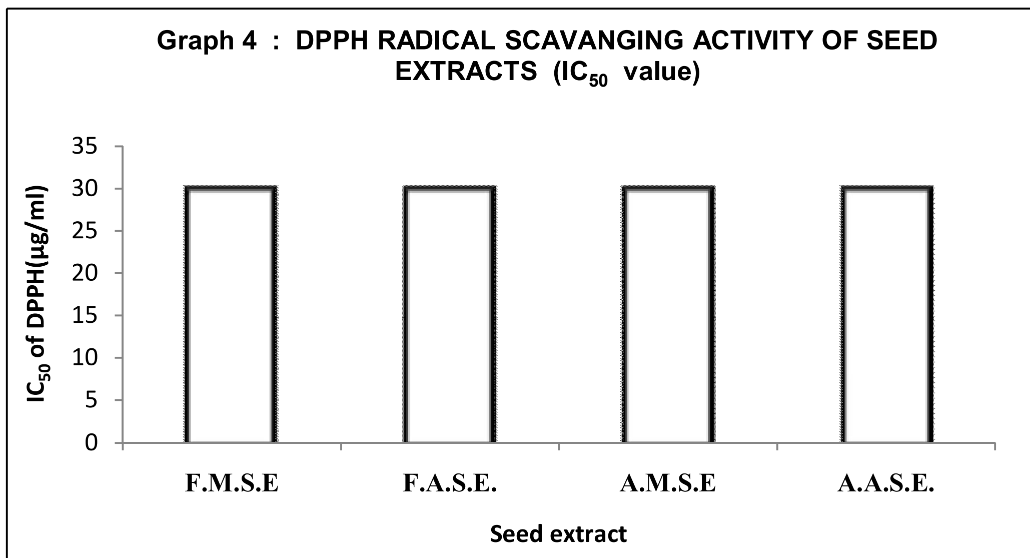
Graph 2 and 3 shows the dose – response curve of DPPH radical scavenging activity of fennel seed extract (FASE and FMSE) and ajwain seed extract (AASE and AMSE) respectively as compared with ascorbic acid. It was observed that FASE and AMSE had higher activity, less than ascorbic acid . At a concentration of 240 µg/ml, the scavenging activity of FMSE reached 78.45%, FASE with 71.27%, AASE with 82.87% and AMSE with 83.42%. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. Though the DPPH radical scavenging abilities of the extracts were less than those of ascorbic acid (100%) at same concentration, the study showed that the extracts have the proton – donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. Concentration of the sample necessary to decrease initial concentration of DPPH• by 50% (IC_{50}) under the experimental condition was calculated. Therefore lower value of IC_{50} indicates a higher antioxidant activity [23]. The experimental data indicate that methanol and aqueous extract of fennel and ajwain seed displayed the similar DPPH• scavenging effect at 30 g/ml (graph 4) . The radical scavenging activity of the extracts could be related to the nature of phenolics and their hydrogen donating ability [18] .

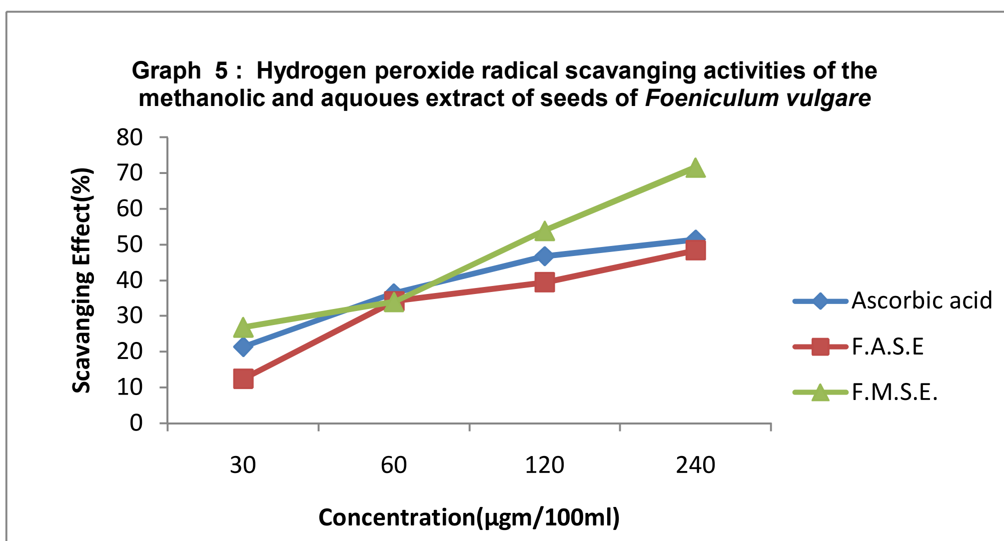
Hydroxyl radical scavenging activity

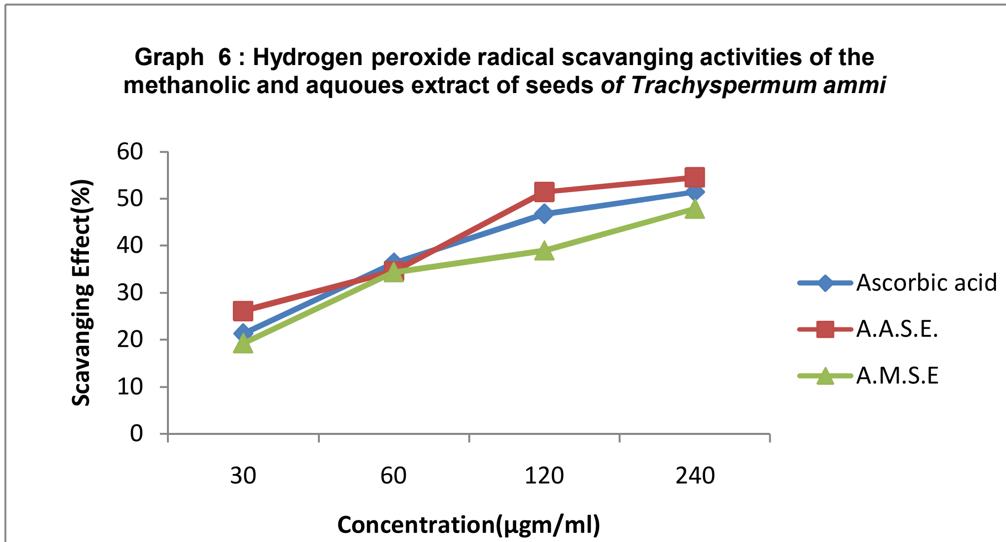
Scavenging of OH- is an important antioxidant activity because of its very high reactivity which can easily cross the cell membranes at specific sites, react with most biomolecules and furthermore cause tissue damage and cell death. Thus, removing OH- is very important for the protection of living systems [24] . Graph 4 and 5, shows the OH- scavenging effect of fennel seed extract and ajwain seed extracts respectively at different dose levels (30, 60, 120 and 240 g/ml). All the extracts generally registered good hydroxyl radical scavenging activity in a concentration dependent manner. Among them, the methanolic extract of fennel seeds showed the highest OH- scavenging potential (71.61% at 240 g/ml concentration) (graph 7) . The ability of the fennel and ajwain seed extracts to quench hydroxyl radicals seems to be directly related to the prevention of propagation of the process of lipid peroxidation, and good scavengers of active oxygen species thus reducing the rate of chain reaction.

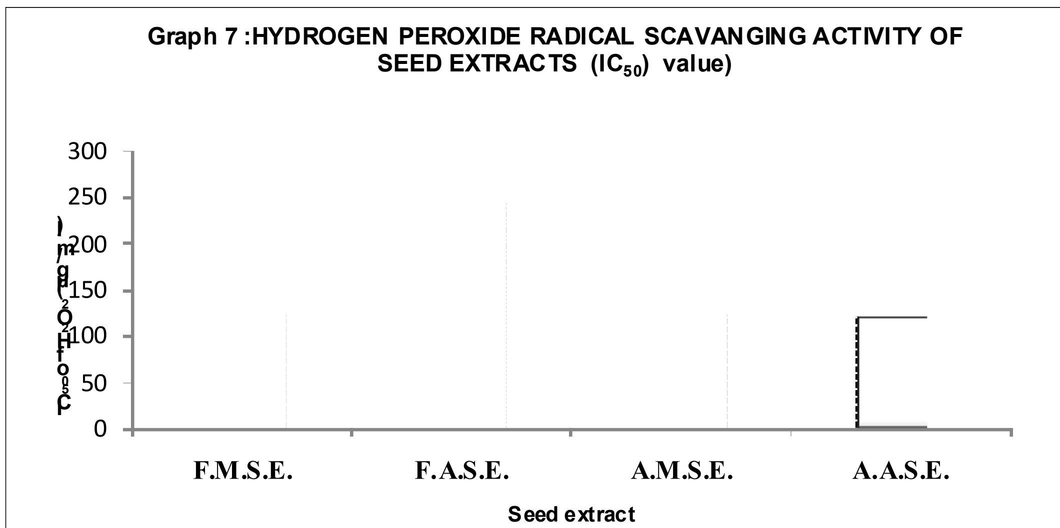


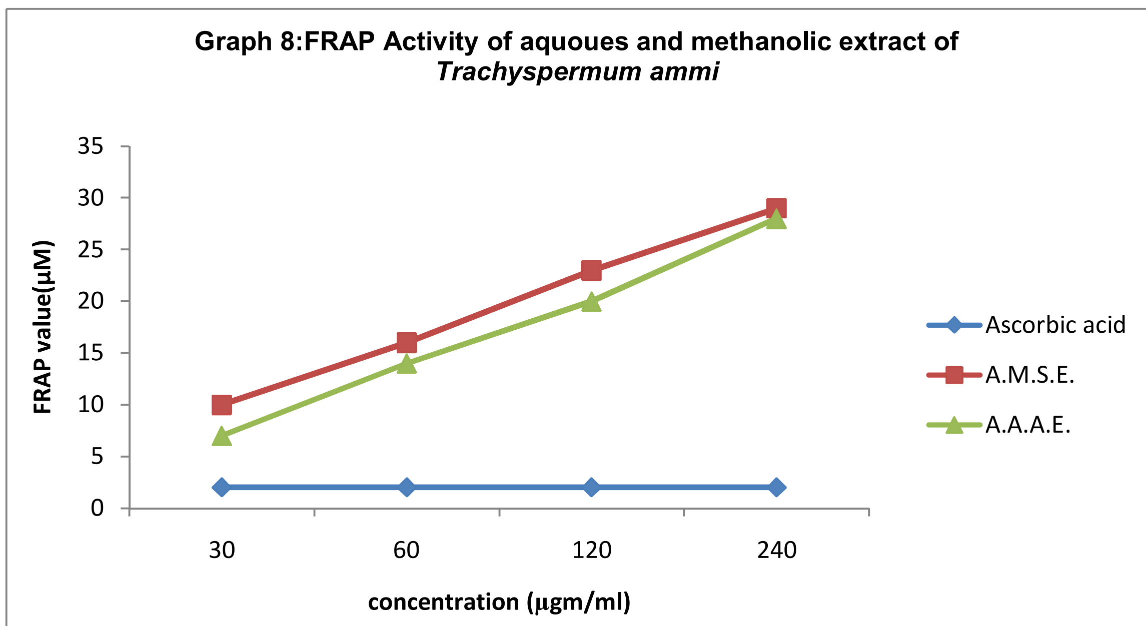

Graph 3 : DPPH radical scavanging activities of the methanolic and aquoues extract of seeds of *Trachyspermum ammi*
Scavanging Effect(%)
120
100
80
60
40
20
0
30
60
120
240
Concentration(µgm/ml)
Ascorbic acid
A.M.S.E

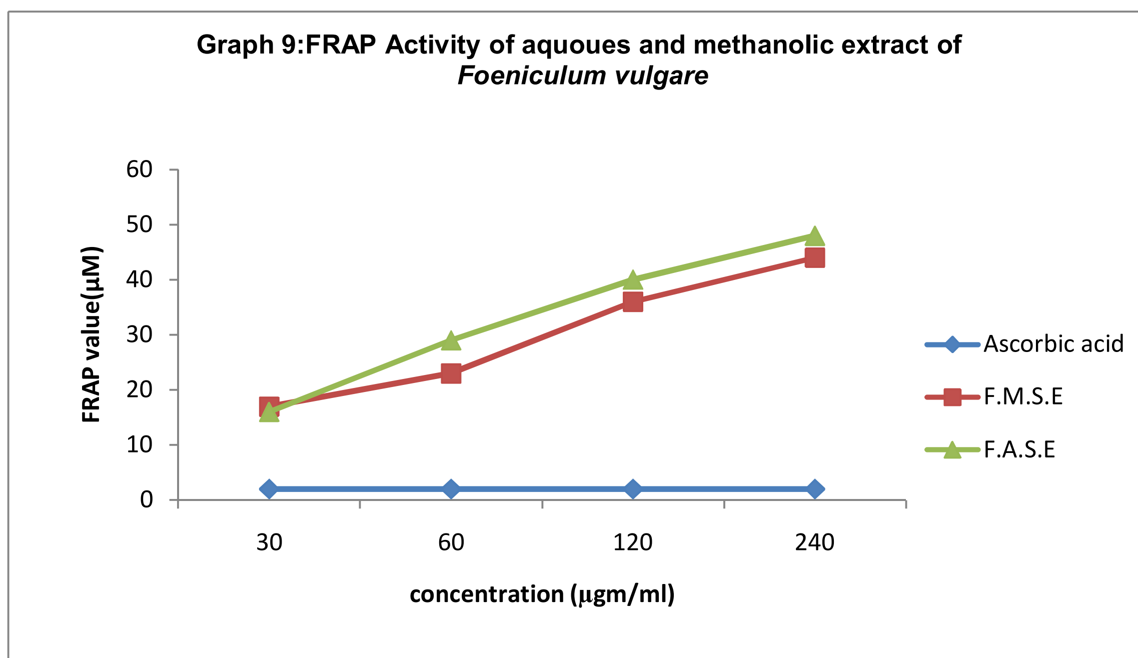

Graph 4 : DPPH RADICAL SCAVANGING ACTIVITY OF SEED EXTRACTS (IC_{50} value)
IC_{50} of DPPH(µg/ml)
35
30
25
20
15
10
5
0
F.M.S.E
F.A.S.E.
A.M.S.E
A.A.S.E.
Seed extract


Graph 5 : Hydrogen peroxide radical scavanging activities of the methanolic and aquoues extract of seeds of *Foeniculum vulgare*
Scavanging Effect(%)
80
70
60
50
40
30
20
10
0
30
60
120
240
Concentration(µgm/100ml)
Ascorbic acid
F.A.S.E
F.M.S.E.


Graph 6 : Hydrogen peroxide radical scavanging activities of the methanolic and aquoues extract of seeds of Trachyspermum ammi
Scavanging Effect(%)
60
50
40
30
20
10
0
30
60
120
240
Concentration(µgm/ml)
Ascorbic acid
A.A.S.E.
A.M.S.E


Graph 7 :HYDROGEN PEROXIDE RADICAL SCAVANGING ACTIVITY OF SEED EXTRACTS (IC50) value)
300
250
200
150
100
50
0
F.M.S.E.
F.A.S.E.
A.M.S.E.
A.A.S.E.
Seed extract


Graph 8:FRAP Activity of aquoues and methanolic extract of Trachyspermum ammi
FRAP value(µM)
35
30
25
20
15
10
5
0
30
60
120
240
concentration (µgm/ml)
Ascorbic acid
A.M.S.E.
A.A.A.E.



Reducing ability (FRAP) assay

The reducing ability of the extracts were in the range of 7 - 48 m Fe (II)/g (Graph 8 and 9). The antioxidant potentials of the methanol and aqueous extracts of seeds of ajwain and fennel were estimated from their ability to reduce TPRZ-Fe (III) complex to TPTZ-Fe (II). The FRAP values for the methanol and aqueous extracts of the seeds of fennel and ajwain were significantly more than that of ascorbic acid. The ferric reducing/antioxidant power (FRAP assay) is widely used in the evaluation of the antioxidant component in dietary polyphenols. Antioxidant activity increased proportionally to the polyphenol content. According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species [25].

CONCLUSION

The phytochemicals derived from plants serve as valuable sources for isolating and characterising the lead molecules with specific functions .This approach assist in identifying the compounds that shows bioactivity. It has been suggested that the compounds that posses antioxidant activity, can inhibit the occurrence of diseases because they can scavenge the free radicals or induced antioxidant enzymes. Due to the phytoantimutagens present in these plants their seed extracts show inhibition to formation of free radicals.

The results of the present study indicate that aqueous and methanol extracts of ajwain and fennel seeds are high in phenolic contents and these extracts exhibit strong antioxidant activities. Further studies are needed to explore the potential phenolics compound(s) from these plants and *in vivo* studies are needed for better understanding their mechanism of action.

REFERENCES

1. Crastes , D.P. (1990). *Ann Biolo Clin* ; 48 : 323.
2. Ames , B . N. (1983) . Dietry carcinogens and anticarcinogens. *Science* , 221:1256-1263.
3. Halliwell , B. (2000) . The antioxidant paradox. *Lancet* ; 355: 1179-1180.
4. Velioglu , Y.S., Mazza , G., Gao , L., Oomah , B.D. (1998) . Antioxidant activity and total phenolics in selected fruits , vegetables and grain products. *J Agric Food Chem* ; 46: 4113-4117.
5. Kaur , C., Kapoor , H.C. (2002). Antioxidant activity and total phenolic content of some Asian vegetables. *Int J Food Sci Tech* ., 37(2): 153-162.
6. Celik , I. and Isik, I. (2008) . *Nat. Prod. Res,* 22: 66-75 .
7. Javidnia , K., Dastgheib , L., Mohammadi Samani, Nasiri, S. (2003) . A.: *Phytomed*, 10: 455-458 .
8. Faudale , M., Viladomat , F., Bastida , J., Poli , F., Codina,C. (2008) . *J Agric Food Chem*, 56:1912-1920 .
9. Wettasinghe , M., Bolling , B., Plhak , P., Parkin , K., (2002). Screening for phase II enzyme inducing and antioxidant activities of common vegetables. *J. Food Sci*. 67 : 2583 -2588.

10. Anonymous : Pharmacopiea of India .(1996) III Edition. Ministry of Health and Family Welfare, Govt. of India , New Delhi.
11. Vargas, V.M.F., Motta, V.E.P., Alice, C.B., Guidobono, R.R., Henriques , J.A.P. (1989) : *REV Bras Farm*, 70:65-67 .
12. Sofowora , E.A. (1993). Medicinal plants and Traditional medicine in Africa. John Willey and Sons, *Chichester*, p. 178.
13. Trease G.E., Evans , W.C., (1989). Pharmacognosy. 13th edn. Bailliere Tindall . London, p. 833.
14. Oyedemi , S. O. , Bradley , G. and Afolayan , A. J. (2010) . *In -vitro* and *-vivo* antioxidant activities of aqueous extract of *Strychnos henningsii* Gilg . *African Journal of Pharmacy and Pharmacology.*, Vol. 4(**2**) pp. 070-078.
15. Ordon , E.Z., Gomez , J.D., Vattuone , M.A., Isla , M.I. (2006). Antioxidant activities of *Sechium edule* (Jacq). *Food chem.,* 97: 452-458.
16. Kumaran , A., Karunakaran , R.J. (2007). *In vitro* antioxidant activities of methanol extracts of *Phyllantus* species from India. Lebens-Wiss technologie. 40: 344 - 352.
17. Sun , J.S., Tsuang , Y.W., Chen , J.J. , Huang, W.C., Hang , Y.S., Lu, F.J . (1998). An ultra - weakchemiluminescence study on oxidative stress in rabbits following acute thermal injury. *Burns* 24: 225-231.
18. Shimada , K.K., Fujikawa , K.Y., Nakamura , T. (1992) . Antioxidative properties of xanthone on autoxidation of soybean oil in cyclodextrin . *J. Agric. Food Chem.* ,40: 945-948.
19. Naik, S.R. (2003) . *Indian Drugs* ; 40: 501.
20. Benzie , F.F. and Strain , J.J. (1999). Ferric Reducing/ Antioxidant Power Assay: Direct Measure of Total antioxidant Activity of Biological Fluids and Modified Version for Simultaneous Measurement of Total Antioxidant Power and Ascorbic Acid Concentration. *Methods in enzymology*. vol. 299:15-23.
21. Zheng , W., Wang S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *J. Agric .Food Chem.* 49**,** 5165–5170.
22. Djeridane , A., Yousfi , M., Nadjemi , B., Boutassouma , B., Stocker , P., Vidal , N. (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem*. 97*,* 654-660.
23. Kandhasamy , S., Jince M. J., Dharmar , R.K., Sellamuthu , M. (2010) . In vitro antioxidant characteristics of different parts of *Melothria maderaspatana* (l.) Cogn. *Int J Pharmacy and Pharm Sci*, Vol 2, Issue 3, 117-123.
24. Yang , J., Guo, J., Yuan, J. (2008) . In vitro antioxidant properties of rutin. Lebensm. *Wiss. Technol.* ; 41: 1060-1066.
25. Oktay , M., Gulcin, I., Kufrevioglu , O. I. (2003). Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. *Lebensm .Wiss.Techol.* 36**,** 263–271.