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Analysis of anti-oxidant properties of ajwain (*Trachyspermum ammi* L) seed extract

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ABSTRACT

Total phenolics, flavonoids content and antioxidant activity of seed extracts of ajwain genotype Ajmer Ajwain-1 and Ajmer Ajwain-2 was analyzed. Total essential oil content from three replicate of var. Ajmer Ajwain-1 was measured 4.94 % while var. Ajmer Ajwain-2 yielded 3.93 % essential oil. The total oil recovered from var. Ajmer Ajwain-1 was 7.66 % while in var. Ajmer Ajwain-2 the oil recovery was 11.5% which is significantly higher than Ajmer Ajwain-1. Methanol extract of both the varieties showed significantly higher amount of phenolics and flavonoids as compared to hexane. Extract similarly, antioxidant properties as measured by % scavenging of DPPH (1, 1-Diphenyl-2-picrylhydrazin) free radicals was found more in methanol extract of both the varieties. There was positive correlation between total phenolic content, total flavonoid content and antioxidant activity. Higher phenolic and flavonoids content showed high anti-oxidant activity. From the present work, it could be suggested that ajowain has potential for the use as source of natural anti-oxidant and this property is directly related with amount of total phenols and flavonoids. Also the solvent used for extraction is very important for effective extraction of the plant constituents.

Keywords: Ajowain (*Trachyspermum ammi* L), Anti-oxidant activity, Flavonoids, Phenolics, Seed extract.

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INTRODUCTION

Free radicals due to environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress, cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins. Oxidation process is one of the most important routs for producing free radicals in food, drugs and even living systems. Due to depletion of immune system natural antioxidants in different maladies, consuming antioxidants as free radical scavengers may be necessary (Halliwell,15; Kuhnan, 18; Kumpulainen and Salonen, 19; Younes, 30). Currently available synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinon and gallic acid esters, have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury. Besides well known and traditionally used natural antioxidants from tea, wine, fruits, vegetables and spices, some natural antioxidant (e.g. rosemary and sage) are already exploited commercially either as antioxidant additives or a nutritional supplements

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(Schuler, 24).

It has been mentioned that antioxidant activity of plants might be due to their phenolic compounds (Cook and Samman, 8). Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (Frankel, 10). Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (Gryglewski *et al.,* 13).

Seed spices group comprises all those annuals whose dried fruit or seeds are used as spices. The seed spices are aromatic vegetable products of tropical origin which are primarily used for seasoning, flavouring and imparting aroma to the food and beverages. They are characterized by pungency, strong odour, sweet or bitter taste. Out of the seed spices grown in India, the most important are coriander, cumin, fennel and fenugreek and put in the category of major seed spices while few other seed spices like ajwain, dill, nigella, celery and aniseed constitute minor seed spices group. Most of the states in India grow one or more seed spices, but major growing belt spread from arid to semi arid regions covering large area in Rajasthan and Gujarat.

Apart from being a potential exportable commodity and source of foreign income all seed spices are well known for their medicinal value since centuries in traditional medicinal systems like Ayurveda and Unani medicine. These medicinal uses of spices and herbs have been used to cure right from common cold to diabetes, from cough to cancerous tumors.

Among different seed spices listed above ajwain (*Trachyspermum ammi*) is important crop used both as spice and condiments in most Indian kitchen preparations. Ajwain with its characteristic aromatic smell and pungent taste is widely used as a spice in curries. Its seeds are used in small quantities for flavouring numerous foods, as preservatives, in medicine and for the manufacture of essential oil for ultimate use in perfumery (Pruthi, 21). In Indian system of medicine, *T. ammi* is administered as a household remedy for stomach disorders, a paste of crushed fruits is applied externally for relieving colic pains; and a hot and dry fomentation of the fruits applied on chest is used as a common remedy for asthma (Anonymous, 3).

In the present study medicinal properties of ajwain was evaluated by analyzing total oil content, volatile oil content, total phenolic content, flavonoid content and antioxidant properties of seed extract.

MATERIALS AND METHODS

Seeds of ajwain (*Trachyspermum ammi* L) varieties Ajmer Ajwain-1 (AA-1) and Ajmer Ajwain-2 (AA-2) were obtained from NRCSS, Ajmer. Obtained seeds were cleaned and used for preparing seed extract.

Total oil was extracted using Accelerated Solvent Extraction System (Dionex India Pvt. Ltd.). Thirty gram seeds were utilized for the estimation. Essential oil from seeds was estimated using all glass Clevenger apparatus (Clevenger, 7), utilizing 25-30 g seed.

Seed extracts were prepared using 30 gm dried seeds which were ground to fine powders by milling. The resulting materials were extracted with methanol and hexane using Accelerated Solvent Extraction system. Final concentration was adjusted to 5mg ml^{-1} of seed material. These diluted extracts were used for determination of the total phenol and flavonoid concentration, as well as antioxidant properties.

Total phenol concentration was determined using a Folin-Ciocalteu assay, as described by Amin *et. al.,* (2) with slight modification. An aliquot of 0.1ml extract (5 mg ml^{-1} in respective solvent) was taken in a test tube and made the volume 1ml by adding solvent. After this, 3ml of 10% sodium carbonate and previously 10-fold diluted Folin-Ciocalteu reagent was added to the mixture. The mixture was allowed to stand at room temperature for 90 minutes and absorbance was measured at 710 nm. Gallic acid was used as the standard phenol. The amount of phenolic content was calculated by using the standard curve of gallic acid prepared with respective solvent having R^2 value ranging from 0.96-0.99 and was expressed as mg Gallic Acid Equivalents/g (mg GAE / g) of seed material.

Total flavonoid concentration was determined by using previously reported method by Chang *et al.*, (5) with slight modification. An aliquot of 1 ml of suitably diluted sample was taken in a test tube and 100 µl aluminum chloride (1M) solution was added carefully from the side wall of the test tube followed by addition of 100 µl potassium acetate. The total volume was made 4 ml by adding 2.8 ml of solvent in the test tube. After 30 minute incubation of reaction mixture at room temperature stable yellow color was developed. Absorbance was measured at 517 nm. Quercetin was used as the standard flavonoids. The amount of flavonoid was calculated by using the standard curve of quercetin prepared with respective solvent having R^2 value ranging from 0.96-0.99 and was expressed as mg Quercetin Equivalents/g (mg QE / g) of plant material.

The antioxidant activity of each extract was evaluated on the basis of its activity in scavenging the stable DPPH (1, 1-Diphenyl-2-picrylhydrazin) radical, using a slight modification of the method described by Shimada *et al.,* 24. Each extract was diluted in methanol/ Hexane to give at least 5 different concentrations. An aliquot of 1, 1.5, 2, 2.5 ml of the extract of each concentration was mixed with 1 ml of 1 M DPPH. The mixture was then homogenized and left to stand for 30 min in dark. The absorbance was measured at 517 nm against a blank of methanol using a spectrophotometer. DPPH solution plus methanol was used as control and Butyl hydroxyl toluene (BHT) was used as a standard reference synthetic antioxidant with R^2 value ranging from 0.95-0.99. Results were expressed as a mean standard deviation from three replicate measurements.

The percent scavenging effect was calculated as follows:

Scavenging effect (%) =

$$\frac{A_{517} \text{ of control} - A_{517} \text{ of extracts}}{A_{517} \text{ of control}} \times 100$$

RESULTS AND DISCUSSION

Total essential oil content from three replicate of var. Ajmer Ajwain-1 was measured 4.94 % while var. Ajmer Ajwain-2 yielded 3.93 % essential oil. It is known that genetic constitution and environmental condition influence the yield and composition of volatile oil produced by medicinal plants (Ramezani *et. al.,* 22, Omidbaigi, 19).

In present study oleoresin was extracted by accelerated solvent extraction system which is completely mechanized and controlled by micro processors hence, recovers more than 98% total oil content of the material with consumption of comparatively less amount of organic solvent. The total oil recovered from var. Ajmer Ajwain-1 was 7.66 % while in var. Ajmer Ajwain-2 the oil recovery was 11.5% which is significantly higher than Ajmer Ajwain-1.The oleoresin varies considerably in composition since the proportion of volatile oil, fatty oil and other extractives depend on cultivar, fruit quality, processing methods and solvent.

The results of TPC and TFC analysis of different seed extracts of two genotype of ajowain are presented in Table 1.

Methanol extract of both the varieties showed less variation in TPC being observed 0.891 and 0.888 mg GAE/g seed in var. Ajmer Ajwain-1 and Ajmer Ajwain-2 respectively. Hexane extract contained significantly less TPC being observed 0.373 and 0.345 mg GAE/g seed in Ajmer Ajwain-1and Ajmer Ajwain-2 genotypes respectively. TFC was also more in methanol extract of both the varieties being higher (0.088 mg QE/ g seed) in AA 1 than AA 2 (0.067 mg QE/g seed). Hexane extract contained less TFC (0.64 mg QE/g seed in both Ajmer Ajwain-1 and Ajmer Ajwain-2). Methanol is found suitable solvent than hexane for extraction of TPC and TFC from ajowain seeds.

Different organic solvents have different polarity and therefore have different nature to extract the compounds. Methanol is best known solvents for non fatty compounds while Hexane is used to extract lipids and oils from plant samples. Tangkanakul *et.al.*, (26), Souri *et.al.* (24), Parichat and Artiwan (19) used methanol extract for total phenol content measurement in fenugreek and other plant species while Kaur and Kapoor (15) used ethanol extract for measurement of antioxidant activity and total phenol content of some Asian vegetables. In present study, however, maximum phenolic contents were observed in methanol extract.

The seed extract of both the organic solvents were evaluated for total antioxidants, free radical scavenging percentage and EC_{50} value.

Total antioxidant was found more in hexane extract of both the varieties. Var. Ajmer Ajwain-1 having slightly more antioxidant than Ajmer Ajwain-2 but in methanol extract it was more in Ajmer Ajwain-2 than Ajmer Ajwain-1. The scavenging % was higher (66.95%) in hexane extract than methanol extract (64.88%) in var Ajmer Ajwain-1 but in var. Ajmer Ajwain-2 scavenging % was higher (68.07%) in methanol extract than hexane (56.91%). EC_{50} value of both the extract was almost same in both varieties, however it was higher in hexane extract (0.007) than methanol extract (0.003)

When free radical scavenging % of both seed extracts were correlated with total phenolic and flavonoid content, there seem a reasonable relationship between these two. In both the extracts scavenging percentage considerably reduced in hexane extract as total phenolic and flavonoid content reduced as compared to methanol extract.

The presence of different antioxidant components in plant tissues especially fruits and vegetables make it relatively difficult to measure each antioxidant component separately. Therefore several methods (Al-Saikhon *et al*., 1; Cao *et al.*, 4; Furuta *et al*., 10; Gazzani *et al*., 11; Vinson *et al*., 27; Kahkonen *et al.*, 14; Chu *et al.*, 6; Tang *et al*., 25) have been developed in

recent years to calculate the total antioxidant activity of biological samples. These workers have also tried different extraction mediums to ensure the maximum extraction of the available anti-oxidants from the samples (Kahkonen *et al*., 14). In the present study, we used methanol and hexane.

While measuring antioxidant activity and total phenolic content of some Asian vegetables Kaur and Kapoor (15) categorized different crops as high, moderate and low phenolic contents vegetables group and correlate this with antioxidant activity. In present study, we observed a clear relationship between total phenolic content, total flavonoid content and antioxidant activity (Fig 1, 2). Higher phenolic and flavonoids content showed high anti-oxidant activity in ajowain seed extracts.

From the present work, it could be concluded that ajowain has potential for the use as source of natural anti-oxidant and this property is directly related with amount of total phenols and flavonoids. The extraction of these compounds also depends upon the solvent used for extraction. Hence, solvent is very important for effective extraction of the plant constituents. Further, studies are needed for the isolation and identification of the active component in the extract.

Table 1. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of seed extract in different organic solvent and two genotype of Ajwain

Ajwain varieties	Total Flavonoid Content (mg GAE g^{-1} seed)		Total Phenolic Content (mg QE g^{-1} seed)	
	Methanol extract	Hexane extract	Methanol extract	Hexane extract
AA 1	0.088	0.064	0.891	0.373
AA 2	0.067	0.064	0.888	0.345
SEm (±)	0.003	0.000	0.007	0.020
CD 0.05	0.013	0.002	0.028	0.079
CV %	9.305	1.685	1.800	12.479

Table 2. Antioxidant activity of seed extract in methanol and hexane of two genotype of ajwain expressed as mg BHT equivalent/g seed

Ajwain varieties	Total Antioxidant (mg BHT E g^{-1} seed)		Scavenging %		EC_{50} (mg BHT E g^{-1} seed)	
	Methanol extract	Hexane Extract	Methanol extract	Hexane Extract	Methanol extract	Hexane Extract
AA 1	0.05040	0.1261	66.9572	64.8817	0.00314	0.00785
AA 2	0.05949	0.1062	68.0780	56.9116	0.00364	0.00777
SEm (±)	0.00935	0.000556	3.28750	0.28000	0.00041	0.000001
CD 0.05	0.05690	0.003388	20.0040	1.70380	0.00250	0.000008
CV %	29.4817	0.83049	8.565184	0.783064	20.9908	0.029926

Fig 1. Relationship between antioxidant activity with phenolic and flavonoid content of ajwain var Ajmer Ajwain- 1

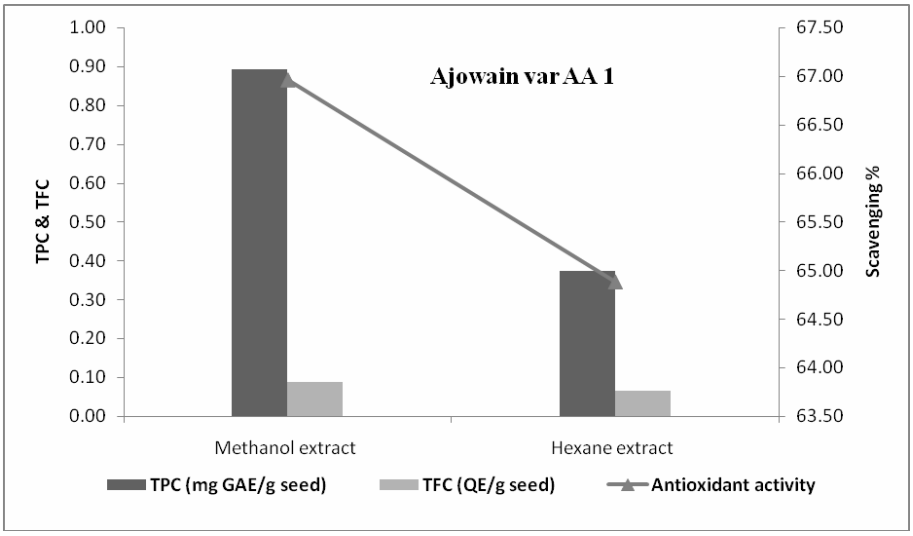
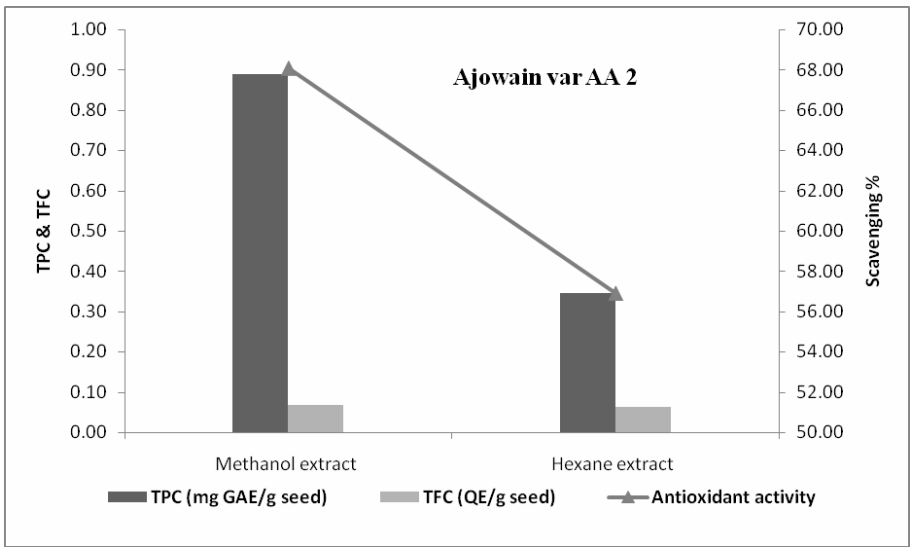


Fig 2. Relationship between antioxidant activity with phenolic and flavonoid content of Ajowain var Ajmer Ajwain- 2.



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