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Analysis of medicinally important compounds and anti-oxidant activity in fixed and essential oil of dill (*Anethum graveolens* L.) genotypes

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ABSTRACT

An experiment was conducted for estimation of medicinal properties and antioxidant activity in fixed and essential oil of sill (*Anethum graveolens* L.) genotypes. Total phenolic content (TPC), total flavonoid contents (TFC), total antioxidant activity content (TAC), scavenging percentage and EC_{50} was measured in oleoresin and essential oil of dill seeds extracted in methanol and DMSO. Genotype AD-2 showed higher TPC and TFC in oleoresin and essential oil as compare to AD-1. Oleoresin showed more TPC and TFC than essential oil in both varieties and solvents. Maximum antioxidant contents and scavenging percentage was observed in oleoresin of AD-2 extracted in menthol. There is no marked difference in EC_{50} value of oleoresin and essential oil from both the genotypes.

Key words : Dill, Antioxidant activity, flavonoids, Phenol.

INTRODUCTION

Dill is an annual herb. Its leaves and seeds are used as seasoning and essential oil extracted from leaves and seeds also used in chewing gums, candies and pickles. Dill leaf consumption could lower the risk of cancer (Yang *et al*. 17) and reduce the level of cholesterolaemia (Lankey *et al*. 6). Moreover, dill leaf, seed and their essential oil could provide good antioxidant activities (Singh et al. 12). Antioxidants act as radical scavengers, inhibit lipid peroxidation and other free radical-mediated processes and are able to protect the human body as well as processed foods from oxidative damage attributed to the reaction of free radicals. The use of synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ) in foods is discouraged due to their perceived carcinogenic potential and safety concerns (Liu and Yao, 7).

Currently, the use of plant-based natural antioxidants, such as flavonoids and phenolic acids and tocopherols in foods, as well as preventive and therapeutic medicine, is gaining much recognition. Such natural substances are believed to exhibit anti carcinogenic potential and offer diverse health-promoting effects because of their antioxidant attributes. (Iqbal et al. 4). Hence, present investigation was taken with the objectives of evaluate genotypic variation in medicinally important compounds and compare total phenolic content, flavonoid and anti oxidant activity of different seed extracts of dill.

MATERIAL AND METHODS

Seeds of two released varieties of Dill (AD 1 and AD 2) were obtained from seed store of NRCSS, Ajmer. The dried seeds (50 gm) were ground to fine powders separately by conventional grinding machine. Fine ground seed powder was taken for volatile oil extraction using all glass Clevenger apparatus. Fixed oil extracted by soxhlet's method. The resulting materials were extracted successively with methanol and DMSO. All the extracts were centrifuged. This process was repeated twice and supernatants were pooled for further analysis. Final concentration was adjusted to 100 ppm of seed extract. These diluted extracts were used for determination of the total phenol and flavonoid concentration, as well as antioxidant activities.

Estimation of total phenol concentrations

Total phenol concentrations were determined using a Folin-Ciocalteu assay, as described by Amin (1). A 0.1ml aliquot of 100 ppm crude seed fixed and essential oil was taken in a test tube and 0.75 ml of Folin-Ciocalteu reagent was added which was previously diluted 10-fold with distilled water. The mixture was allowed to stand at room temperature for 5 minutes and 3 ml of 10 % sodium carbonate followed by 10 ml distilled water was added. After standing for one minute at room temperature, absorbance was measured at 725 *nm*. Gallic acid was used as the standard phenol. The amount of phenolic content was calculated by using the standard curve of Gallic acid having R^2 value ranged from 0.97 and was expressed as mg Gallic acid equivalents/100 ppm crude seed extract.

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Estimation of total flavonoids content

Total flavonoid content was determined using previously reported method by Chang et al. (3), with slight modification. One ml of sample from 100 ppm crude extract was taken in a test tube and 100µl aluminum chloride (1M) solution was added carefully from the side wall of the test tube followed by addition of 100µl 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 ranged from 0.96-0.99 and was expressed as mg Quercetin equivalents/100 ppm crude seed extract.

Antioxidant assays

The antioxidant activity of crude seed 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 (11). An aliquot of 1 ml of crude seed extract was mixed with 1 ml of 1 M DPPH. The mixture was then homogenized and left to stand for 30 min in the 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 ranged 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:

$$\text{Scavenging effect (\%)} = \frac{A_{517}\text{ of Control} - A_{517}\text{ of Extract}}{A_{517}\text{ of Control}} \times 100$$

The EC_{50} value for each sample defined as the concentration of the test sample leading to 50% reduction of the DPPH concentration was calculated from the non linear regression curve of the test extract.

RESULTS AND DISCUSSION:

Perusal of Table 1 revealed the results of total phenolic contents (TPC) and Total flavanoids content (TFC) in both genotypes of Dill. TPC was more in oleoresin as compare to essential oil in both extraction medium as well as in both genotypes. Maximum TPC was observed in methanol extracted oleoresin of genotype AD-2 (7.05 mg g^{-1} Gallic Acid Equivalent/100 ppm extract), while minimum TPC was observed in essential oil of genotype AD-1 extracted in DMSO (0.72 mg g^{-1} Gallic Acid Equivalent/100 ppm extract). Over all Genotype AD-2 showed significantly higher phenolic contents both in oleoresin and essential oil.

Table 1. Total Phenolic Content (TPC) and Total Flavanoid Content (TFC) in oleoresin and essential oil of dill genotypes.

Treatment	Total Phenolic Content (TPC) (mg g^{-1} GAE/100 ppm)		Total Flavanoid Content (TFC) (mg g^{-1}QE/100 ppm)	
	AD 1	AD 2	AD 1	AD 2
Fixed oil (Methanol)	6.99	7.05	16.98	19.70
Essential oil (Methanol)	3.73	2.27	4.71	6.34
Fixed oil (DMSO)	6.34	4.56	0.98	1.01
Essential oil (DMSO)	0.72	0.96	0.75	0.77
SEM	0.12	0.22	0.10	0.33
CD 0.05	0.43	0.75	0.63	1.13
CV %	4.82	10.05	1.30	9.65

Table 2. Antioxidant Content, Scavenging and EC_{50} value in fixed oil and essential oil of dill genotypes

Treatment	AO Content (mg BHT E/100 ppm)		Scavenging (%)		EC_{50}	
	AD 1	AD 2	AD 1	AD 2	AD 1	AD 2
Fixed oil (Methanol)	6.512	7.526	52.746	58.391	6.173	6.444
Essential oil (Methanol)	3.944	5.509	32.560	44.857	6.057	6.140
Fixed oil (DMSO)	1.232	1.345	12.521	12.354	6.014	6.025
Essential oil (DMSO)	0.986	1.089	11.254	14.012	6.012	6.004
SEM	0.411	0.413	3.578	3.564	0.514	0.513
CD 0.05	1.421	1.431	12.380	12.421	1.779	1.770
CV %	12.112	12.212	13.145	13.212	14.351	13.989

Total flavanoids content (TFC) is higher in fixed oil as comparison to essential oil in both extraction medium as well as in both genotypes. Maximum TFC was observed in fixed oil of genotype AD-2 (19.70 mg g^{-1} QE/ 100 ppm) when extracted in methanol while minimum TFC was observed in essential oil of genotype AD-1 extracted in DMSO (0.75 mg g^{-1} QE/100 ppm). Genotype AD-2 showed higher flavanoids contents both in fixed oil and essential oil. Difference found significantly in all treatments.

Total antioxidant content is differ significantly in different genotypes. In fixed oil, maximum antioxidant contents observed in methanolic extract of genotype AD 2 (7.526 mg BHT E/100 ppm) while minimum TAC observed in essential oil of AD 1 dissolve in DMSO (0.986 mg BHT E/100 ppm).

Data presented in Table 2 indicated that scavenging percentage is differ significantly in different genotypes. Maximum scavenging percentage was observed in fixed oil of AD 2 (58.391 %) while minimum scavenging percentage observed in essential oil of AD 1 (11.252 %). There is no observable difference between EC_{50} value of genotypes, fixed oil and essential oil. Maximum EC_{50} value observed in fixed oil of AD 2 (6.444) while minimum EC_{50} value observed in essential oil of AD 2 (6.004).

Phenolic compounds widely exist in plants are bioactive substances. It is well known that they are highly effective antioxidants (Shahidi and Naczk, 9, Shahidi and Wanasundara, 10 and Tapiero et al., 16). Flavonoids could provide strong antioxidant activities associated with their capacity to scavenge free radical and terminate radical chain reactions (Bors *et al*. 2). .

Different organic solvents have different polarity and therefore have different nature to extract the compounds. Methanol and Ethanol are best known solvents for non fatty compounds while Hexane and Dichloromethane are used to extract lipids and oils from plant samples. Tangkanakul *et.al.*, (15), Souri *et.al.,* (14), Parichat and Artiwan (8) used methanol extract for total phenol content measurement in fenugreek and other plant species while Kaur and Kapoor (5) used ethanol extract for measurement of antioxidant activity and total phenol content of some Asian vegetables. Skerget et al. (13) indicated that phenols, proanthocyanidins, flavones and flavonols may be responsible for the effective antioxidant properties of the dill flower extract. In present study it was found that fixed oil extracted from both the genotype of dill showed high phenolic, flavonoids and antioxidant content as compare to essential oil. Dill seeds may be a vital source of natural anti oxidant.

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