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Phytochemical screening and antioxidant activity of *Anethum graveolens* L. seed extracts

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Abstract

Anethum graveolens L. commonly known as dill is an annual medicinal herb belonging to family Umbelliferae. In this study, the possible antioxidant properties of different extracts of dill seeds were investigated using various *in vitro* models viz. DPPH, hydroxyl radical, nitric oxide radical, superoxide radical scavenging methods and ferric reducing antioxidant power (FRAP) assay at various concentrations viz. 0.05, 0.1, 0.25, 0.5 and 1.0 mg/ml respectively. Results revealed that the methanol extract was most effective as compared to other tested extracts. Total phenolics and flavonoids content were found to be higher in methanol extract as compared to others tested extracts. IC₅₀ of all the extracts were found to be less than 1.5 mg/ml. All the tested extracts exhibited lower antioxidant potential as compared to ascorbic acid used as standard. Phytochemical screening confirmed the presence of reducing sugars, saponins, anthraquinones, cardiac glycosides, terpenoids and phlobatanins. Dill seed can be exploited as a source of natural antioxidant.

Keywords: *Anethum graveolens* L., antioxidant activity, dill seed, phytochemicals, solvent extracts

Introduction

Essential oils are complex mixture of low molecular weight compounds extracted by steam distillation, hydro distillation or solvent extraction methods [1]. Essential oils are produced by plants as volatile secondary metabolites for their own need other than for nutrition. Plant essential oils exhibit several applications in field of health, agriculture, cosmetic and food industries [2]. For controlling various diseases essential oil plays a vital role. Free radicals such as reactive oxygen species (ROS) produced by continuous exposure, air pollution, ionization radiation and smoking have the capacity of oxidizing lipids, proteins and DNA, leading to serious skin disorder including high pigmentation, premature ageing and skin cancer. The oxidation of lipids is delayed or inhibited by antioxidants as they cause inhibition of the initiation or propagation of oxidizing chain reactions [3]. There are numerous approaches reported [4] in protection against the oxidative damage caused by ROS and among them, foremost is through high consumption of antioxidants. Plant based natural antioxidants are preferred due to human safety [5]. Presence of flavonoids, phenolics, terpenes, saponins, tannins and cardiac glycosides is responsible for good antioxidant activity [6]. *Anethum graveolens* L. (Umbelliferae), commonly known as dill, is native to Mediterranean countries and South-eastern Europe. The dill seeds have a strong spicy odour, acting as flavouring agent in the food industry for salads, sauces, soups, tea, sea foods and especially in pickles [7, 8]. Dill seed essential oil is used in perfumery to aromatize cosmetics, detergents, soaps, as carminative and for control of flatulence, colic and hiccups in infants and children [9]. Pharmacological effects of dill such as antibacterial and antimycobacterial have been reported [10]. In present studies phytochemical analysis and antioxidant activity of various extracts of dill seeds were evaluated using different *in vitro* models.

Materials and methods

Preparation of extracts

The different extracts of dill seed were obtained by Soxhlet extraction using hexane, dichloromethane and methanol as the solvents separately. 100 g powdered dill seeds were extracted with 500 mL of hexane, dichloromethane and methanol in separate round bottomed flasks on heating bath. The solvent was removed by distillation on rotary evaporator and the crude extracts were stored at 4 °C. The stock solution (2 mg/ml) of each compound was prepared by dissolving 20 mg of compound in 10 ml methanol. The required dilutions of 1.0, 0.5, 0.25, 0.1 and 0.05 mg/ml were subsequently made from the stock solution using methanol as solvent.

Phytochemical screening

The presence of phytochemicals such as flavonoids, phenolics, reducing sugar, saponins, steroids and tannins in extracts was detected using standard protocols [11]. The various tests carried out, their procedure, observation and inference is tabulated in Table 1.

Estimation of total phenolic and flavonoid content

Total phenolic content was determined according to Follin-

Ciocalteu method [12]. The amount of total phenols present in the extracts was calculated from the standard curve of gallic acid and results were expressed as milligrams of gallic acid equivalent per gram. Total flavonoid content was estimated by aluminium chloride [13] method. The amount of total flavonoids present in the extracts was calculated from the standard curve of quercetin and results were expressed as milligrams of quercetin equivalent per gram.

Table 1: Procedures for the phytochemical analysis

Phytochemicals	Procedure	Observation	Inference
Flavonoids	1 ml of the extract + 1% of aluminium chloride	Intense yellow colour	Present
Phenolics	0.5 g extract in water + 5% ferric chloride	Dark green colour	Present
Reducing sugars	2 ml extract + Fehling's solution A and B in equal volume	Red precipitate	Present
Saponins	50 mg of extract boiled with 10 ml distilled water + shook vigorously + 2-3 drops of olive oil	Emulsion	Present
Antraquinones	100 mg extract boiled with 1% hydrochloric acid + 3 ml benzene + 2 ml 10% ammonia solution	Pink, violet or red colour	Present
Cardiac glycoside	1 mg extract + 2 ml glacial acetic acid + few drops of ferric chloride solution	Brown ring at interface	Present
Terpenoids	5 ml extract + 2 ml chloroform + 3 ml sulfuric acid	Reddish brown colour at interface	Present
Phlobatannins	50 mg extract was boiled with 1% hydrochloric acid	Red precipitate	Present

In vitro Antioxidant assay

DPPH radical scavenging method

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging was determined using method prescribed by Liyana and

Shahidi [14]. The absorbance of the mixture was measured at 517 nm using spectrophotometer. Ascorbic acid was used as a standard. All tests were performed in triplicate. The percentage inhibition was calculated using the formula.

$$\text{Percentage inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

IC₅₀ values were calculated from the graph plotted between scavenging capacity versus concentration. Lower IC₅₀ indicated higher antioxidant capacity.

Hydroxyl radical-scavenging method

Hydroxyl radical scavenging activity was determined by

$$\text{Percentage inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

IC₅₀ values were calculated from the graph plotted between scavenging capacity versus concentration.

Nitric oxide radical scavenging method

Nitric oxide radical scavenging was assessed by method given by Green *et al.* [16]. The absorbance of chromophore formed

$$\text{Percentage inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

IC₅₀ values were calculated from the graph plotted between scavenging capacity versus concentration.

Ferric reducing antioxidant power (FRAP) assay

Ferric reducing antioxidant power was determined by method specified by Benzie and Strain [17]. Tested compounds (0.2 ml) were allowed to react with 2.8 ml of the FRAP solution for 30 min in the dark condition. Readings of the coloured product (ferrous tripyridyltriazine complex) were taken at 593 nm. Results were expressed in terms of µg Fe (II)/g dry mass. A standard curve of Fe²⁺ ion was also obtained using FeSO₄.

deoxyribose degradation method [15]. The absorbance of the chromophore formed was read at 532 nm against blank prepared in similar way. Control was also run parallel, in which no test material was added. The percentage inhibition was determined by comparing the absorbance values of tested components and control using the formula.

was read at 548 nm against blank. The percentage inhibition of nitric oxide generation was measured by comparing the absorbance value of tested components and control using the formula. IC₅₀ values were calculated from the graph plotted between scavenging capacity versus concentration.

Superoxide radical scavenging method

Measurement of superoxide anion scavenging activity of each extract was determined by PMS-NADH method [18]. Superoxide radicals were generated in 3.0 ml of Tris-HCl buffer (16 mM, pH 8.0), which contained 78 µM β-nicotinamide adenine dinucleotide (reduced form, NADH), 50 µM nitroblue tetrazolium (NBT), 10 pM phenazine methosulfate (PMS) and dill seed extract of varying concentrations. The colour of superoxide radicals and NBT was detected at 560 nm. Ascorbic acid was used as the standard in this experiment.

Results and discussion

Phytochemical analysis

Phytochemical analysis showed the presence of phenolics and flavonoids in addition to reducing sugars, saponins, anthraquinones, cardiac glycosides, terpenoids and phlobatanins (Table 2). Previous phytochemical analysis of aqueous, methanol, acetone, petroleum ether and chloroform leaf extracts of *Holoptelea integrifolia* and *Celestrus*

emarginata revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, coumarins, quinines, cardiac glycosides, xanthoproteins, glycosides, steroids, phenols, resins, carboxylic acid group in varying concentrations [19]. Similar results were reported for the presence of saponins, tannins, steroids, flavonoids, glycosides and terpenoids in leaves, stems, roots, *in vitro* callus and regenerated leaves of *A. graveolens* [20].

Table 2: Phytochemical analysis of various extracts of dill seeds.

Chemical Constituent	Methanol extract	Dichloromethane extract	Hexane Extract
Flavonoids	+	+	+
Phenolics	+	+	+
Reducing sugar	+	-	+
Saponins	+	+	-
Anthraquinones	-	-	-
Cardiac glycoside	-	-	-
Terpenoids	+	+	+
Phlobatannins	+	-	-

(+) indicates the presence and (-) indicates absence

Total phenolic and flavonoid content

The extraction yield in various solvents showed significant differences due to difference in polarity of different compounds present in the dill seeds. The extraction yield increased with increase in polarity of the solvent. The antioxidant activity can be related to total phenolic and flavonoid content of the plant material. Phenolic compounds have an important role in absorbing and neutralizing free radicals, quenching singlet, and triplet oxygen or decomposing peroxides [21]. Total phenolic content was expressed as milligrams equivalent of gallic acid per gram of dry extract (mg GAE/g). Results of quantitative analysis revealed that total phenolic content in extracts of dill seed varied from 7.06 to 19.09 mg GAE/g. Methanol extract was most effective amongst all the tested extracts having a high content of total phenols 19.09 mg GAE/g followed by dichloromethane and hexane extract 12.28 and 7.06 mg GAE/g, respectively. The total phenol and flavonoid content of extract were greatly affected by polarity of the solvent. More polar the solvent used, more was the total phenolic and

flavonoid content, hence the antioxidant potential [22]. As reported earlier, the total phenolic content in different extracts of *Marrubium peregrinum* L. ranged from 27.26 to 89.78 mg GAE/g. The significant linear correlation was confirmed between the values for the total phenolic content and antioxidant activity of plant extracts. The high content of phenolic compounds indicated that these compounds contribute to the antioxidant activity [23]. Flavonoids help to protect the cells from oxidation [24]. Flavonoid content was expressed as milligrams equivalent of quercetin per gram of dry extract (mg QE/g). The total flavonoid content in dill seed extracts varied from 2.45 to 5.07 mg QE/g. Methanol extract showed higher content of total flavonoids (5.07 mg QE/g) followed by hexane and dichloromethane extracts which contained moderate levels of flavonoids (2.45 and 3.99 mg QE/g, respectively). This is in agreement with earlier findings which revealed that total phenolic and flavonoid content in different extracts of ajwain seeds depends on the polarity of the extraction solvent and increased with increase in polarity of solvent [25].

Table 3: Comparison of yield total phenolic and flavonoid content in different extracts of dill seeds.

Extract	Yield (%)	Total phenols (mg GAE g ⁻¹)	Total flavonoids (mg QE g ⁻¹)
Methanol Extract	1.2±0.7	19.09±0.16	5.07±0.28
Dichloromethane Extract	3.3±0.4	12.28±0.09	3.99±0.62
Hexane Extract	5.7±0.9	7.06±0.24	2.45±0.97

DPPH radical scavenging

1,1-diphenyl-2-picryl-hydrazil radical (DPPH•) assay has been widely used to test the capacity of compounds to act as free radical scavengers for the evaluation of antioxidant capacity. DPPH is a molecule containing stable free radical which can be reduced in the presence of hydrogen donating antioxidants. The change in colour was determined spectrophotometrically [26]. The results of DPPH radical-scavenging activity revealed that methanol extract was found to be most effective in all the tested extracts and antioxidant activity increased with increase in concentration of tested components. The higher activity of methanol extract of dill seed can be related to its high phenolic and flavonoid content. Methanol extract exhibited highest antioxidant activity with IC₅₀ value of 0.30 mg/ml followed by dichloromethane and hexane extracts having IC₅₀ value of 0.46, and 0.95 mg/ml

respectively. However antioxidant activity of the various extracts was found to be lower than that of ascorbic acid (IC₅₀ = 0.08 mg/ml). Similar results were earlier reported for antioxidant activity of different extracts of ajwain seeds [25]. DPPH scavenging activity of ethanolic extract of *Triphala* also increased in a concentration dependent manner compared to ascorbic acid. The overall antioxidant activity was attributed to its polyphenolic and other phytochemical constituents [27]. Earlier DPPH studies on water, ethanol and acetone extracts of dill leaf revealed that water extract (1.93 ± 0.53 mg/ml) showed the highest DPPH• radical scavenging activity, followed by the ethanol extract (4.75 ± 1.35 mg/mL), while the acetone extract revealed the lowest activity (8.95 ± 1.41 mg/ml) [28].

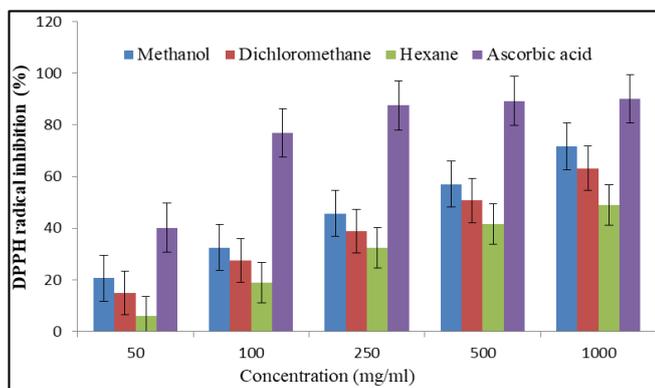


Fig 1: Inhibition effect of the hexane, dichloromethane and methanol extracts of dill seed on DPPH radical scavenging method.

Hydroxyl radical scavenging

Hydroxyl radicals generated in the human body are the major active oxygen species causing lipid peroxidation and tissue injury at sites of inflammation in oxidative stress-originated diseases [29]. Radical was generated by incubating ferric-ethylene diamine tetra acetic acid with ascorbic acid and hydrogen peroxide at pH 7.4 and reacted with 2-deoxy-2-ribose. The potential of the components to quench hydroxyl radicals was directly related to the prevention of propagation of lipid peroxidation [30]. All components exhibited antioxidant activity in a concentration dependent manner as antioxidant activity increased with increase in concentration of tested components. The methanol extract was found to be stronger scavenger of hydroxyl radical than hexane and dichloromethane extracts. At the mentioned concentration, the hydroxyl radical scavenging activity of all extracts was lower than that of standard ascorbic acid. Methanol extract showed highest antioxidant activity with IC₅₀ value of 0.28 mg/ml as compared to dichloromethane and hexane extracts with IC₅₀ value of 0.59 and 1.43 mg/ml respectively. Standard ascorbic acid was found to have an IC₅₀ value of 0.08 mg/ml. As already reported [31] aqueous extracts of dill seeds showed stronger hydroxyl radical scavenging activity with an inhibition percentage of 43.56%.

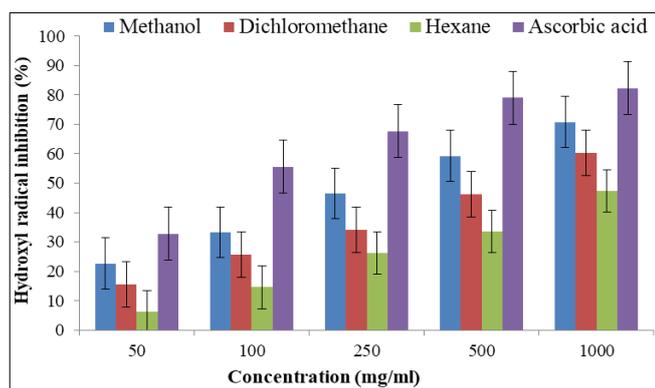


Fig 2: Inhibition effect of the hexane, dichloromethane and methanol extracts of dill seeds on hydroxyl radical.

Nitric oxide radical scavenging

Nitric oxide is a very unstable species and reacting with oxygen molecule to produce stable nitrate and nitrite which can be estimated using Griess reagent. Nitric oxide radical scavenging activity of methanol, dichloromethane and hexane extracts of dill seeds varied in concentration dependent manner and increased with increase in concentration. All extracts showed good nitric oxide radical scavenging activity.

As compared to standard ascorbic acid (IC₅₀ = 0.08) methanol extract was most effective as nitric oxide radical scavenger (IC₅₀ = 0.24 mg/ml) followed by dichloromethane extract (IC₅₀ = 0.39 mg/ml) whereas, hexane was least effective (IC₅₀ = 1.28 mg/ml). The IC₅₀ of the stem extract of *Actaea racemosa* (black cohosh) was lower (1.46 µg/ml) than the standard (1.66 µg/ml) in scavenging nitric oxide radical. These result indicated that the extracts could either serve as a free radical inhibitors or scavengers using their proton donating ability or could act as the primary antioxidants [32].

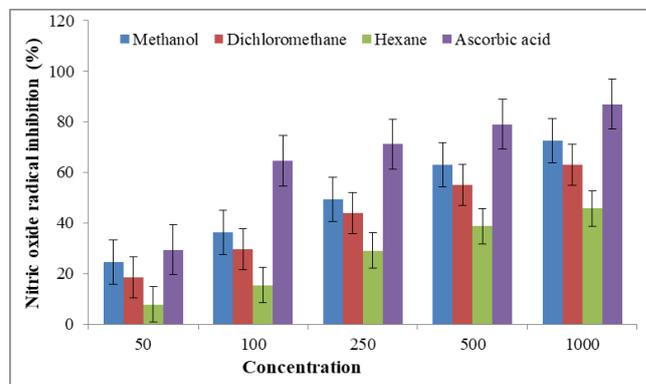


Fig 3: Inhibition effect of the hexane, dichloromethane and methanol extracts of dill seeds on nitric oxide radical.

Ferric reducing antioxidant power

The reducing ability of all the tested components was expressed in terms of µg Fe²⁺/g, compared with ferrous sulfate standard. The reducing power (absorbance at 593 nm) of the tested components was concentration dependent which increased with increase in concentration. Reducing power of methanol extract was significantly higher than that of other tested extracts with FRAP value of 599.70 µg Fe (II)/g at 1.0 mg/ml concentration. Dichloromethane extract also showed moderate reducing power with FRAP values of 270.20 µg Fe (II)/g at 1.0 mg/ml concentration. However least ferric reducing antioxidant power was exhibited by hexane extract with FRAP value of 198.2 µg Fe (II)/g at 1.0 mg/ml concentration. FRAP value is most likely due to reducing ability of compounds like carvone and limonene present in the dill seeds [33].

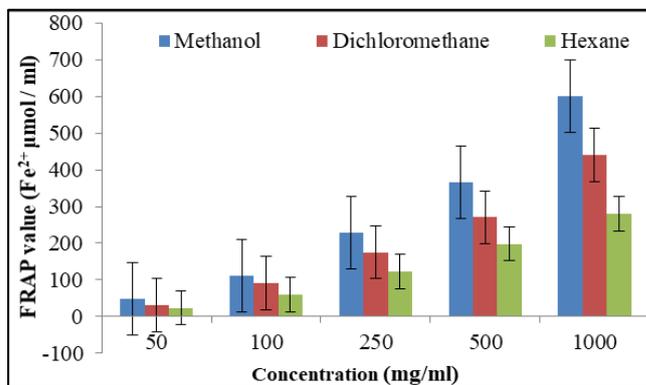


Fig 4: Reducing power of the hexane, dichloromethane and methanol extracts of dill seeds.

Superoxide radical scavenging

The superoxide radicals were generated in a PMS-NADH system and assayed by the reduction of NBT. Superoxide radical scavenging activity of methanol, dichloromethane and hexane extracts of dill seeds varied widely and increased with increase in concentration levels. Methanol extract showed

highest superoxide radical scavenging activity ($IC_{50} = 0.24$ mg/ml). Moderate activity was possessed by dichloromethane extract ($IC_{50} = 0.36$ mg/ml) followed by hexane extract of dill seeds ($IC_{50} = 1.27$ mg/ml). All the extracts showed good antioxidant activity but less than standard ascorbic acid ($IC_{50} = 0.08$ mg/ml). The methanolic extract of 70% *Caesalpinia crista* leaves examined for different ROS scavenging activities showed IC_{50} values of 440 ± 0.1 , 24.9 ± 0.98 , 33.72 ± 0.85 , 61.13 ± 3.24 and 170.51 ± 4.68 μ g/ml for hydroxyl, superoxide, nitric oxide, singlet oxygen and hypochlorous acid, respectively. Results indicated that antioxidant activity of methanol extract of *C. crista* leaves was due to the presence of bioactive antioxidants in addition to phenolics and flavonoids [34].

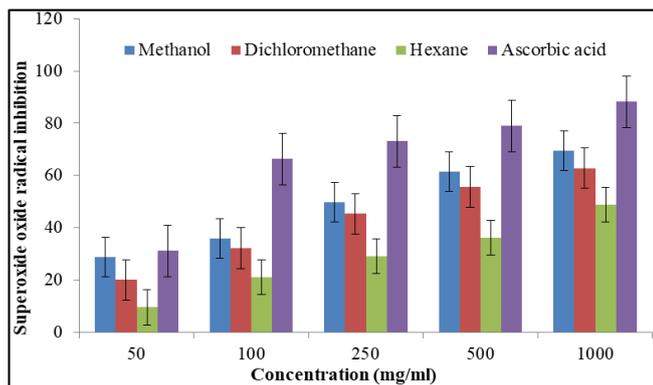


Fig 5: Inhibition effect of the hexane, dichloromethane and methanol extracts of dill seeds on superoxide radical.

Conclusion

The undesired side effects of synthetic antioxidants attract the interest of food producers and consumers in finding ingredients of natural origin. Different solvent extracts of dill seed evaluated for its antioxidant potential. The methanol extract was found to be stronger scavenger of free radical than all other tested extracts. The higher activity of methanol extract can be related to its higher phenolic and flavonoid contents. The total phenolic and flavonoid content of extract were greatly affected by polarity of the solvent. More the polarity of the solvent used, more was the total phenolic and flavonoid content hence the antioxidant potential. Phytochemical screening also indicated the presence of reducing sugars, saponins, anthraquinones, cardiac glycosides, terpenoids and phlobatanins. These results indicated the possible application of dill seeds in food and pharmaceutical industry as a safer alternative to synthetic antioxidants. The percentage inhibition of hexane, dichloromethane and methanol extracts of dill seed followed the order.

Hexane extract > Dichloromethane extract > Methanol extract.

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