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In Vitro Antioxidant Potential and Phytochemical Analysis of *Anethum graveolens* Stem Extract: A Comprehensive Study

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Abstract: *Anethum graveolens*, belongs to the family Apiaceae. The aim of the present work was to screen the antioxidant potentiality of the stem extract of *Anethum graveolens*. The proteins were isolated from *Anethum graveolens* stem and analyzed for their antioxidant activity by DPPH radicals where ascorbic acid was used as standards at different concentrations. The results are expressed as percentage (%) inhibition exhibited by the test extract and the standard antioxidant. Total phenol, flavonoid, and alkaloid were found to be highest in methanolic extract of *Anethum graveolens*. The percentage inhibition of radical's activity increased with increase in concentration of crude protein. *Anethum graveolens* extract is comparable with standard antioxidant. The results showed that the *Anethum graveolens* proteins have good antioxidant activity when compared to standards. The results of the present investigation concluded that methanolic extract of *Anethum graveolens* stem extract (MEAGS) demonstrated concentration dependent *in vitro* anti-oxidant activity due to the presence of bioactive component(s).

Keywords: Antioxidant, Free radical, Oxidative stress, *Anethum graveolens*, DPPH, Phytochemicals

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Introduction

Oxidative stress plays an essential role in the pathogenesis of chronic diseases such as cardiovascular diseases, diabetes, neurodegene-rative diseases, and cancer (Al-Oqail and Farshori, 2021). Long term exposure to increased levels of pro-oxidant (smoke, environ-mental pollution) factors can cause structural defects at a mitochondrial DNA level, as well as functional

alteration of several enzymes and cellular structures leading to aberrations in gene expression. The modern lifestyle associated with processed food, exposure to a wide range of chemicals (environmental pollution), lack of exercise and higher levels of dietary xenobiotics results in the generation of free radicals which are harmful to human health (Vertuani, 2004).

The term 'free radical' includes related reactive species such as 'excited states' that lead to free radical generation or those species resulting from free radical reactions. In general, free radicals are very short lived, with half-lives in milli-, micro- or nanoseconds (Devasagayam *et al*., 2004). Free radical, also referred to as reactive oxygen species or ROS. There are several different types of free radicals (e.g. hydroxyl radical, superoxide radical, nitric oxide radical and lipid peroxyl radical), which also come from cigarette smoke, environmental pollution, radiation, and UV light. Excessive free radicals can cause tissue damage and hypoxia, over exposure to environmental factors (smoking, UV radiation, and pollutants). When the production of damaging free radicals exceeds (a condition known as Oxidative stress), body's antioxidant defenses to detoxify them,. It is said that oxidative stress is imbalance between generation of free radicals and antioxidant defense capacity of the body and is closely associated with aging of cell and etiology of various diseases including cancer, cardiovascular diseases, diabetes and diabetic complications (Banerjee *et al*., 2005).

Antioxidants is a molecule stable enough to donate an electron to rampaging free radical and neutralize it, thus reducing its capacity to cellular damage. These antioxidants delay or inhibit cellular damage mainly through their free radicals scavenging property (Chahal *et al.*, 2017). Some of antioxidants are produced during normal metabolism in the body. Other antioxidants are found in the diet. Although there are several enzyme systems within the body that scavenge free radicals, the principal micronutrient antioxidants are vitamin E (α-tocopherol), vitamin C (ascorbic acid), Flavonoids and β- Carotene. In market several synthetic antioxidants are available which includes Butylatedhydroxytoluene (BHT), Butylated-hydroxy anisole (BHA), Tertiary butylhydro-quinone (TBHQ), Propyl gallate which are commonly used. Synthetic antioxidants have been widely used, however, safety issues have been raised over time (Fernandez-Panchon *et al*., 2008)

. There are several published studies indicating a relationship between long term intake of synthetic antioxidants and some health issues, such as skin allergies, gastrointestinal tract problem and in some cases increased risk of cancer. High doses of synthetic antioxidants may cause DNA damage and induce premature senescence. Butylatedhydroxy anisole (BHA) and Butylatedhydroxytoluene (BHT) have already been found responsible for adverse effects on the liver and for carcinogenesis in animal's studies. So tendency to replace these synthetic antioxidants with natural ones has been increasing due to minimum adverse effects (Jamuna *et al*., 2011).

Nowadays the whole world return towards herbal products and to adopt more natural way of life. People prefer natural food, herbal medicine and natural curing practices for healthy lifestyle. There are plenty of synthetic antioxidants which are used to prevent oxidative stress but antioxidants formulated from herbal plants are safe and effective. Majority of antioxidant capacity of herbal product or natural products is due to presence of phytoconstituents such as Polyphenols like Flavonoids, Tannins along with vitamin A, B, C and E and Cartenoids. Antioxidants also used in pharmaceutical formulation which prevent the oxidation during storage and increase shelf life of the pharmaceutical formulation (Heath *et al*., 2005).

. *Anethum graveolens* (family Apiaceae), is indigenous to southern Europe. It is an annual herb growing in the Mediterranean region, central and southern Asia. Now it is cultivated widely throughout the world (Goodarzi *et al*., 2016). It is used traditionally as a popular aromatic herb and

spice that has a very long history of use going back to more than 5,000 years. It was used as a remedy for indigestion and flatulence and as milk secretion stimulant (Al-Snafi, 2014). Moreover, it is used as an anti-convulsion, anti-emetic, anti-cramp (in children), as a wound healer and to increase the appetite and strengthen the stomach (Hajhashemi and Abbasi, 2008). *Anethum graveolens* contains essential oils, fatty oil, moisture (8.39%), proteins (15.68%), carbohydrates (36%), fiber (14.80%), ash (9.8%), furanocoumarin, polyphenols and mineral (Fig. 1).

To the best of our knowledge there exists no report on *in vitro* anti-oxidant activity of stem of *Anethum graveolens.* Hence, the present study was undertaken for preliminary phytochemical investigation and screening of anti-oxidant activity of stem of *Anethum graveolens stem* extract by DPPH method.

Materials and Methods

Plant material

The plant sample was identified and authenticated by Dr. Ramachandra Naik M, Department of Botany, S.B Arts and KCP Science College, Vijayapur, Karnataka, India. *Anethum graveolens* stem were collected locally and dried at room temperature.

Preparation of extract

The *Anethum graveolens* stem were ground to coarse powder and then extracted with methanol by Soxhlet's extraction method. Thereafter, the extract was concentrated using rotary flash evaporator. The yield of the extract obtained was 11 %. The obtained crude extract was stored in airtight container in refrigerator 10 °C for further studies.

The metanolic extract of *Anethum graveolens* stem (MEAGS) was suspended in 2% gum acacia in distilled water and subjected to the following studies:

1. Preliminary phytochemical screening.
2. *In vitro* anti-oxidant activity of methanolic extract of *Anethum graveolens* stem by DPPH method.

In vitro anti-oxidant activity of methanolic extract of Anethumgraveolens stem by DPPH method

DPPH radical scavenging activity

Prepared 0.05 mM solution of DPPH by adding 9.8 mg of DPPH in 50 ml of ethanol and incubated it at normal room temperature for 2-3 h. Stock solution of 1 mg/ml of the drug/plant extract was prepared in distilled water and diluted to get various concentrations (10-100 µg/ml). The concentration can thus be further increased depending upon the results (Leelaprakash *et al*., 2011).

Working solution was prepared by mixing 10 ml of the stock solution to 40 ml of ethanol. 1 ml of the working solution was mixed with l ml of various extract/drug. Concentrations (10-100 µg/ml) were prepared in a final volume of 1 ml. Incubated the mixture for 20 min at room temperature. After 20 min absorbance of reaction mixtures was recorded at 517 nm. In all the experiments, distilled water served as blank and reaction mixtures without plant extract/drug dilutions (1 ml of working solution) served as control samples The % DPPH radical scavenging activity was calculated using the following formula (Meenakshi *et al*., 2009):

%Inhibition of DPPH radical scavenging activity = (Abs of control - Abs of samples)/Abs of control x 100

Statistical analysis

All data are expressed as mean ± standard deviation of five replicate (n=5). The significance of the experimental observation was checked by Student's t-test and $P < 0.05$ was considered as statistically significant when compared to relevant controls.

Results and Discussion

The presence and absence of phytochemicals present in *Anethum graveolens* extract have been shown in Table 1. The percentage inhibition of DPPH radicals increased with increase in concentrations of crude protein of *Anethum*

Table 1: Preliminary phytochemical screening of MEAGS

S. No.	Phytochemical Constituents	Inference
1	Carbohydrate	+
2	Proteins	+
3	Alkaloids	+
4	Phenolic compounds	-
5	Tannins	+
6	Steroids	-
7	Flavonoids	+
8	Glycosides	+

+ Indicates presence, - Indicates absence

Table 2: Absorbance and % Inhibition of DPPH scavenging activity of the crude extract of *Anethum graveolens* and Standard antioxidant at different concentrations

Group	Absorbance	% Activity
Ascorbic acid (µg / ml)		
5	0.400	58.634
10	0.146	84.901
15	0.325	66.390
20	0.384	60.279
***Anethumgraveolens* stem Extract (µg / ml)**		
5	0.514	46.844
10	0.504	47.880
15	0.541	44.053
20	0.523	45.915

graveolens extract. DPPH assay was based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, which denotes that extract has significant antioxidant potential (Table 2).

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH reacts with O_2 to form nitrite ion. Aqueous extract inhibited nitrite formation in concentration dependent manner. Antioxidant nature of the extract complete with oxygen to react with nitric oxide. It was observed that, percentage (%) inhibition was increased with the increase in concentration of the extract of crude protein of *Anethum graveolens* at various concentrations (Table 2).

The percentage inhibition of superoxide radical scavenging activity of crude protein of *Anethum graveolens* was increased with increasing concentrations.

In the present study, the evaluation of *in vitro* anti-oxidant activity and preliminary phytochemical investigation of methanolic extract of *Anethum graveolens* stem was carried out by DPPH method.

The proteins were isolated from *Anethum graveolens* stem were analyzed for their antioxidant activity by DPPH radicals where ascorbic acid was used as standards at different concentrations. The results are expressed as percentage (%) inhibition exhibited by the test extract and the standard antioxidant.

The percentage inhibition of radical's activity increased with increase in concentration of crude protein *Anethum graveolens* extract which is comparable with standard antioxidant. The results

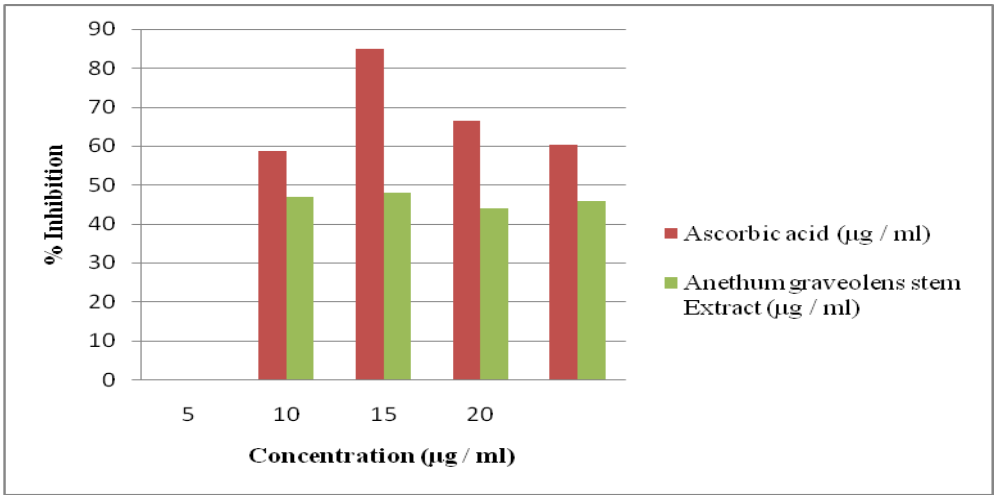

Fig. 1: % Inhibition of DPPH scavenging activity of the Crude exyract of *Anethum graveolens* and Standard antioxidant at different concentrations.

show that the *Anethum graveolens* proteins have good antioxidant activity when compared to standards.

The percentage inhibition of DPPH radicals increased with increase in concentrations of crude protein of *Anethum graveolens* extract. DPPH assay was based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, which denotes the extract has significant antioxidant potential.

Conclusion

The results of the present investigation concluded that methanolic extract of *Anethum graveolens* stem extract (MEAGS) demonstrated concentration dependent *in-vitro* anti-oxidant activity effect due to presence of bioactive component(s). Further research has to be carried out to fractionate and purify the test extract, to isolate the bioactive molecule(s) responsible for the observed anti-oxidant activity.

Conflict of Interest

The authors declare no conflicts of interest.

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