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Bioactivity and Phytochemical Studies of Seed Extracts of *Anethum graveolens* Linn.

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Abstract: Infectious diseases cause enormous morbidity and mortality of the population worldwide every year. The increase in the rate of infectious diseases and bacteria attaining resistance to antibiotics and side effects of some synthetic antibiotics has led to an increased interest in medicinal plants as a better and natural alternative to synthetic drugs. Antibacterial activity of solvent extracts of *Anethum graveolens* L. was evaluated by agar cup diffusion and disc diffusion methods against some common pathogenic bacteria, namely, *Escherichia coli, Klebsiella pneumoniae*, *Bacillus subtilis*, *Bacillus cereus*, *Salmonella typhi,* and *Enterobacter aerogenes.* The antioxidant capacity of different extracts of *Anethum graveolens* was estimated by diphenyl picryl hydrazyl (DPPH), nitric oxide (NO), and hydrogen peroxide. Solvent extracts of *A. graveolens* recorded a good 18-19 mm inhibition zone activity with methanol extract. The MIC of *A. graveolens* methanol extract was recorded in the range 125-1667 μg/ ml for test pathogens. The IC_{50} values showed that methanol extract was nearly potent to butylated hydroxyanisole (BHA) followed by ethyl acetate extracts in all the methods. Methanol extracts of *A. graveolens* possess a broad spectrum of activity against several human pathogenic and potent antioxidant properties. A natural substance obtained from plants that is a part of a daily diet, a nutritional supplement with antimicrobial and antioxidant properties, constitutes a new source of herbal drugs.

Keywords: antibacterial; Minimum Inhibitory Concentration; free radicals; antioxidants; phytochemicals.

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1.Introduction

According to the WHO, infectious diseases are a cause of morbidity and mortality worldwide, accounting for approximately 50% of all deaths worldwide. Although the progress made in the study of bacteria and the control of microorganisms, sporadic incidents of epidemics have emerged due to drug-resistant microbes posing an enormous threat to public health [1]. The increase in the rate of infectious diseases on the population and bacteria attaining resistance to antibiotics and side effects of some synthetic antibiotics has led to an increased interest in medicinal plants as a better and natural alternative to synthetic drugs [2].

Approximately 90% of the body's energy is generated by oxygen**.** Oxidation is a chemical reaction that produces free radicals. Such free radicals produced in chain reactions

may damage cells. Generally, antioxidants play an important role in inhibiting the enzymes that are responsible for damaging healthy cells. The antioxidants with low-molecular weights can safely bind with reactive oxygen free radicals to inhibit the biochemical reaction before damaging the biomolecules. [3]. Several synthetic compounds such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are used as antioxidants, but the use of BHA and BHT is proved to be toxic and carcinogenic [4]

Plants are known for the biosynthesis of a large array of secondary metabolites (SMs), and these metabolites form the basis for various commercial pharmaceutical drugs derived from medicinal plants. The phytoconstituents with biological activities can improve human health [5]. Plant material contains mainly the primary or secondary types of metabolites. These are also referred to as natural products. The plant chemicals having medicinal properties are largely the secondary metabolites derived bio-synthetically from primary metabolites that are not directly involved in plants' growth, development, or reproduction [6]. These secondary metabolites can be classified into several groups according to their chemical classes, such as alkaloids, terpenoids, steroids, phenols, tannins, etc. [7]

India is endowed with a rich wealth of medicinal plants, representing a valuable source of natural products for maintaining human health. A large number of medicinal plants are used in several formulations for the treatment of various diseases caused by microorganisms. Nowadays, the world has shown a great interest in curing diseases using plants/plant-based drugs [8]. In recent years, many possible natural antibacterial agents have been used for several infectious diseases of bacterial and fungal origin [9]. Several plant extracts, natural foods, Vitamin C and E, beta-carotene and tocopherol, and phytochemicals such as flavonoids, isoflavones, phenolics flavones, anthocyanins, coumarins, and lignans are known to possess antioxidant properties [10]. The antimicrobial and antioxidants potential of phytochemicals and their benefits have encouraged the authors to scientifically validate the antibacterial and antioxidant potential of *Anethum graveolens* belonging to the family Apiaceae.

Anethum graveolens belong to the family Apiaceae and order Apiales. It is commonly known as 'Dill', which is cultivated throughout the plains in India but originated in the Mediterranean. It prefers a sandy loam soil of moderate fertility and is cultivated throughout Indian plains. It is an annual, erect, glabrous herb, 30- 90 cm in height, hallows finely grooved stem, striped, dark green and white with bluish spots. Leaves are compound, 2- 3 pinnate with ultimate filiform, bluish-green, and leaf sheath surrounding the stem. Flowers are pale yellow, in a compound umbel, accrescent, terminal, large fruits oblong, dorsally compressed, 3- 4 mm long, 1.5- 3.0 mm broad, ridged narrowly, and winged having two mericarps (Figure 1). The leaves and seeds are extensively used as dietary vegetables, and essential oil is extracted from the seeds. The major constituents of the oil are carvone (30-63%)[11]. The dill leaves are used as a seasoning for soups, sauces, and pickles and used for their carminative and preservative properties [12]. The nutritional composition of *A. graveolens* seed is given in Table 1 [13].

Table 1. The nutritional composition of *A. graveolens* seeds.

Constituents	Approximate composition	Constituents	Approximate composition
Moisture	11.3%	Calcium	498.9 mg/ 100 g
Crude Protein	21.9%	Iron	56.3 mg/ 100 g
Fat	2.4%	Magnesium	196.5 mg/ 100 g
Carbohydrates	31.7%	Potassium	892.1 mg/ 100 g
Crude fibre	5.9%	Sodium	30.2 mg/ 100 g
Ash	10%	Chromium	0.12 mg/100 g

Constituents	Approximate composition	Constituents	Approximate composition
Oleic acid	31.2 mg/ ml	Titanium	0.008 mg/ 100 g
Linoleic acid	9.2 mg/ ml	Molybdenum	0.015 mg/ 100 g
Linolenic acid	0.6 mg/ 100 g	Vitamin C	121.4 mg/ 100 g

A

B

Figure 1. (**A**) *Anethum graveolens* plant; (**B**) *Anethum graveolens* seeds.

2. Materials and Method

2.1. Plant material.

Healthy and clean seeds *Anethum graveolens* of were collected from Mysuru, Karnataka, and used to prepare different solvent extracts.

2.2. Preparation of extracts

Healthy seeds were cleaned thoroughly, shade dried, and powdered with the help of a warning blender. 50 g of the powder was filled in a thimble and extracted successively with petroleum ether, chloroform, ethyl acetate, and methanol in a soxhlet extractor for 48 h. The extracts were concentrated using a rotary flash evaporator and preserved at 5 °C in an airtight bottle until further use. All the extracts were tested for antibacterial and antioxidant activity.

2.3. Human pathogenic bacteria.

Authentic cultures of human pathogenic bacteria viz., *Bacillus cereus* (*B. cereus*) (MTCC 1272), *Bacillus subtilis* (*B. subtilis*) (MTCC 121), *Escherichia coli* (*E. coli*) (MTCC 7410), *Enterobacter aerogens* (*Ent. aerogens*) (MTCC 7325), *Klebsiella pneumoniae* (*Klb. pneumoniae*) (MTCC 7407), *Salmonella typhi* (*S. typhi*) (MTCC 733) and *Staphylococcus aureus* (*Staph. aureus*) (MTCC 7443) served as test bacteria were obtained from Microbial Type Culture Collections (MTCC), Chandigarh, India.

2.4 Antibacterial activity.

2.4.1 Agar cup diffusion assay.

The antibacterial activity of solvent extracts was determined by agar cup diffusion [14] and disc diffusion methods [15]. Cups were made in plates containing 20 ml of nutrient agar media using sterile cork borer (6 mm), and inoculum containing 10^6 CFU/ml of bacteria were spread uniformly over the solid media with a sterile swab moistened with the bacterial suspension. The solvent extracts were reconstituted respectively in sterile distilled water and methanol to a 100 mg/ml concentration. Solvent extracts of 100 µl were placed in the cups made in the inoculated plates. Also, 100 µl of sterilized distilled water and methanol were placed in the cups separately, which served as a negative control, and 100 µl of antibiotic-

containing 20 µg streptomycin (streptomycin sulfate IP, 200 µg/ml) served as a positive control. The plates were incubated for 24 h at 37 °C, and the zone of inhibition, if any around the cups, were measured in mm. For each treatment, four replicates were maintained and repeated twice.

2.4.2. Disc diffusion assay.

The disc diffusion method was carried out according to NCCLS protocol. 100 µl aqueous and solvent extracts were loaded to sterile discs of 6 mm and placed on inoculated nutrient agar in the Petri plates. Also, sterile discs loaded with 100 µl of sterilized distilled water and methanol served as a negative control. Antibiotic disc of streptomycin (20 µg) (streptomycin sulfate IP) served as a positive control. The plates were incubated for 24 h at 37 °C, and zones of inhibition, if any around the discs, were measured in mm. For each treatment, four replicates were maintained and repeated twice.

2.4.3. Minimum inhibitory concentration (MIC).

The MIC was determined in 96 well flat bottom microtiter plates based on microdilution assay, an automated turbidometric and colorimetric method described by Das [16]. Inoculum of the test bacteria was prepared from 24 h old bacterial cultures in sterile/saline water, and turbidity of the suspension was adjusted to 0.5 Mc Farland.

The crude extracts of methanol, ethyl acetate, and chloroform were diluted to the concentration of 100 mg/ml, which served as stock solution. The 96 well plates were filled with 200 µl of nutrient broth and 100 µl of the extract to the first well. A two-fold serial dilution was made along the rows, and final concentrations were 5 to 0.019 mg/ml. A 10 µl inoculum of each test bacteria was added to each well.

The wells containing nutrient broth with inoculum and solvent served as a negative control. The plates were incubated at 37 °C for 24 h, and the optical density was measured at 620 nm using a microplate reader (LT4000, LABTECH Instruments, UK). The lowest concentration that inhibited the visible growth of the bacteria was recorded as the MIC based on the optical density.

The MIC was also confirmed by adding 10 µl of TTC (2, 3, 5-triphenyl tetrazolium chloride, Sigma) dissolved in sterile distilled water (TTC 2 mg/ml) to each well and incubated at 37 °C for 30 min in the dark[17]. Viable organisms reduced the dye to a pink color compound. The lowest concentration at which the color change occurred was taken as the MIC. All MIC tests were repeated thrice.

2.5. Antioxidant assay.

The antioxidant capacity of different extracts of *Anethum graveolens* was estimated by diphenyl picryl hydrazyl (DPPH), nitric oxide (NO), and hydrogen peroxide scavenging methods.

2.5.1. DPPH radical scavenging assay.

The free radical-scavenging activity of the different extracts was measured in terms of hydrogen donating or radical scavenging ability using stable radical DPPH as described by the Blois method [18]. A volume of 1 ml dimethyl sulfoxide (DMSO) was used to dissolve 0.001 g of the studied extracts for the preparation of stock solutions. Variable concentrations such as

20, 40, 60, 80, and 100 μg of the above stock solution were diluted to 2 ml in methanol. A volume of 1 ml methanolic solution of DPPH (0.1 mmol) was added to each of the test solutions. Thoroughly shaken the above mixture and recorded the absorbance of each test solution at 517 nm after 30 min incubation time. A triplicate measurement was made and presented as SD ± mean. Ascorbic acid (AA) and DMSO were used as the standard and negative control, respectively. The capacity of the extracts to scavenge the DPPH radical was calculated using the following equation:

$$DPPH^{\cdot} \text{ scavenging effect (\%)} = [(A_c - A_b)/A_c \times 100]$$

where, A_c is the absorbance of the negative control, i.e., without sample; A_b is the absorbance of the sample.

2.5.2. Hydroxyl radical scavenging assay.

The hydrogen peroxide scavenges activity of these compounds was assayed according to the report by Ruch *et al.* [19]. The Fenton reaction system was used to generate hydroxyl radical in an aqueous media. DMSO (1 ml) solvent was used to prepare the studied extract solution. A volume of 5 ml assay mixture was prepared using the reagents mentioned below: Transfer different volumes of the extract such as 4, 8, 12, 16, and 20 μl into a series of flasks containing Safranin (11.4 μmol), EDTA–Fe(II) (40 μmol), H_2O_2 (1.76 μmol) and diluted to 5 ml with phosphate buffer (0.067 mol, pH 7.4). The absorbance of the formed species was recorded at 520 nm after incubation (37 °C) period of 30 min. Triplicate measurement was made for each using BHA (butylated hydroxyanisole) as a standard, and the values are presented SD ± mean. The scavenging capacity of OH· was determined by employing the below formula:

$$\text{Suppression capacity (\%)} = [(A_o - A_i)/A_o \times 100]$$

where A_0 and A_i are the absorbance of the control and test compound, respectively.

2.5.3. Nitric oxide scavenging assay.

Griess reagent comprises 1% sulphanilamide, 2% phosphoric acid, and 0.1% naphthyl ethylenediamine dihydrochloride.

Test of nitric oxide radical scavenging capacity was determined according to the method of Green *et al*. [20]. The assay is based on nitric oxide (NO) generation from sodium nitroprusside (SNP), and the same was reacted with naphthyl ethylenediamine dihydrochloride in the presence of sulphanilic acid. At physiological pH, the generated nitric oxide from nitroprusside was reacted with oxygen to produce nitrite ion, which can be measured using the cited reagent. In phosphate buffer (pH 7.4), nitroprusside was mixed with different extract concentrations (20, 40, 60, 80 and 100 µg). After incubating (at 25 ºC for 150 min) the mixture, it was treated with a 1.5 ml Griess reagent volume. At room temperature, again, it was incubated for 30 min and diluted with buffer solution(4 ml). The absorbance of the formed purple azo dye was recorded at 546 nm against the reagent blank. Triplicate measurement was made for each with the use of ascorbic acid as a standard, and the values are presented SD ± mean. The following formula was used to calculate the percentage radical scavenging effect.

$$\text{Nitric oxide}^{\cdot} \text{ scavenging effect (\%)} = [(A_c - A_t)/A_c \times 100]$$

A_c= absorbance of control

A_t= absorbance in the presence of the sample of extract

2.6. Phytochemical analysis.

Phytochemical analysis of aqueous and solvent extracts was carried out to detect secondary metabolites by adopting the procedure reported by Harborne [21]. The results are tabulated and discussed.

2.7. Statistical analysis

Statistical calculations were carried out using one-way ANOVA (analysis of variance). The significance of the differences between means was calculated using Tukey's multiple range test under the significance level of $P < 0.05$.

3. Results

3.1. Antibacterial activity of different solvent extracts of A. graveolens against human pathogens.

Solvent extracts of *A. graveolens* recorded a good activity (Figure 2), while chloroform extract had the least activity by cup diffusion method (Table 2). Among the test bacteria *B. cereus, Ent. aerogenes, Staph. aureus, B. subtilis, Klb. pneumoniae* and *S. typhi* recorded a good activity of 18-19 mm inhibition zone with methanol extract, which was slightly less than the standard antibiotics streptomycin and gentamicin, indicating the efficacy of this extract. Antibacterial activity of *A. graveolens* by disc diffusion method was similar to that of cup method. Methanol extract showed maximum activity in the range 17- 19.50 mm, followed by ethyl acetate (10- 16.25 mm) and petroleum ether (7-13.5 mm), while chloroform extract recorded a negligible activity (Table 3). The MIC of *A. graveolens* methanol extracts was recorded in the range 125- 1667 µg/ ml for human pathogens.(Table 4).

Table 2. Zone of inhibition of *A. graveolens* extracts and antibiotics against human pathogenic bacteria by agar cup diffusion method (in mm).

Bacteria	Solvent control	Pet. Ether	Chloroform	Ethyl acetate	Methanol	Streptomycin	Gentamycin
B. cereus	0.00	$13.50{\pm}0.64^{bc}$	$12.25{\pm}0.25^{ab}$	$17.25{\pm}0.47^{b}$	$19.50{\pm}0.64^{a}$	$21.00{\pm}0.40^{bc}$	$24.30{\pm}0.33^{b}$
B. subtilis	0.00	$12.00{\pm}0.40^{cd}$	$09.00{\pm}0.40^{d}$	$16.25{\pm}0.25^{a}$	$18.25{\pm}0.25^{a}$	$20.75{\pm}0.47^{bc}$	$29.30{\pm}0.88^{a}$
E. coli	0.00	$13.75{\pm}0.25^{b}$	$09.25{\pm}0.47^{cd}$	$11.50{\pm}0.28^{a}$	$14.50{\pm}0.28^{b}$	$23.75{\pm}0.47^{a}$	$22.00{\pm}0.57^{b}$
Ent. aerogens	0.00	15.75 ± 0.25^{a}	$10.75{\pm}0.25^{bc}$	$14.75{\pm}0.25^{a}$	$19.75{\pm}0.62^{a}$	$21.25{\pm}0.62^{bc}$	$23.60{\pm}0.88^{b}$
Kleb. pneumoniae	0.00	$13.50{\pm}0.28^{bc}$	$11.75{\pm}0.25^{ab}$	$14.75{\pm}0.28^{a}$	$18.25{\pm}0.25^{a}$	$19.75{\pm}0.62^{c}$	$22.60{\pm}0.33^{b}$
Salm. typhi	0.00	$11.75{\pm}0.25^{d}$	$12.75{\pm}0.25^{a}$	$16.25{\pm}0.25^{a}$	$18.75{\pm}0.25^{a}$	$22.50{\pm}0.28^{ab}$	$23.60{\pm}0.66^{b}$
Staph. aureus	0.00	$13.75{\pm}0.25^{b}$	$08.75{\pm}0.47^{d}$	$15.25{\pm}0.25^{a}$	$19.50{\pm}0.28^{a}$	$19.25{\pm}0.47^{c}$	$30.30{\pm}0.33^{a}$

Values are means of four independent replicates. Figures followed by different letters in columns differ significantly when subjected to Tukey's HSD ($P < 0.05$).

Table 3. Zone of inhibition of *A. graveolens* extracts and antibiotics against human pathogenic bacteria by disc diffusion method (in mm).

Bacteria	Solvent control	Pet. Ether	Chloroform	Ethyl acetate	Methanol	Streptomycin	Gentamycin
B. cereus	0.00	$13.50{\pm}0.47^{a}$	$08.50{\pm}0.50^{b}$	$16.25{\pm}0.25^{a}$	$17.25{\pm}1.74^{ab}$	$19.66{\pm}0.33^{bc}$	$23.75{\pm}0.47^{bc}$
B. subtilis	0.00	$11.50{\pm}0.50^{ab}$	$08.25{\pm}0.25^{b}$	$14.25{\pm}0.47^{b}$	$18.25{\pm}0.25^{a}$	$17.30{\pm}0.33^{d}$	$28.50{\pm}0.50^{a}$
E. coli	0.00	$11.00{\pm}0.57^{ab}$	$07.50{\pm}0.28^{b}$	$10.75{\pm}0.25^{a}$	$14.50{\pm}0.28^{b}$	$23.30{\pm}0.33^{a}$	$22.75{\pm}0.47^{bc}$
Ent. aerogens	0.00	$11.75{\pm}0.25^{ab}$	$09.25{\pm}0.47^{ab}$	$13.50{\pm}0.28^{b}$	$19.75{\pm}0.62^{a}$	$21.30{\pm}0.33^{b}$	$22.25{\pm}0.47^{c}$
Kleb. pneumoniae	0.00	$10.75{\pm}0.25^{abc}$	$08.75{\pm}0.47^{ab}$	$16.25{\pm}0.25^{a}$	$18.25{\pm}0.25^{a}$	$19.00{\pm}0.57^{cd}$	$24.75{\pm}0.25^{b}$
Salm. typhi	0.00	07.00 ± 0.25^{c}	$10.50{\pm}0.25^{a}$	$14.75{\pm}0.47^{ab}$	18.75 ± 0.25^{a}	$20.30{\pm}0.33^{bc}$	$22.25{\pm}0.47^{c}$

Bacteria	Solvent control	Pet. Ether	Chloroform	Ethyl acetate	Methanol	Streptomycin	Gentamycin
Staph. aureus	0.00	09.25±0.25[bc]	08.50±0.28[b]	13.75±0.25[b]	19.50 ± 0.28[a]	17.60±0.33[d]	23.00±0.40[bc]

Values are means of four independent replicates. Figures followed by different letters in columns differ significantly when subjected to Tukey's HSD ($P < 0.05$).

Table 4. Minimum Inhibitory Concentration of Methanol extract against human pathogenic bacteria (in µg/ml).

Plant	*B. cereus*	*B. subtilis*	*E. coli*	*Ent. aerogens*	*Kleb. pneumoniae*	*Salm. typhi*	*Staph. aureus*
A. graveolens	1040	1667	1250	625	833	1660	125
Streptomycine	12.5	25	12.5	6.25	12.5	12.5	6.25
Gentamicin	1.05	0.525	3.10	1.05	3.10	1.05	0.525

Values are mean of three independent replicates.

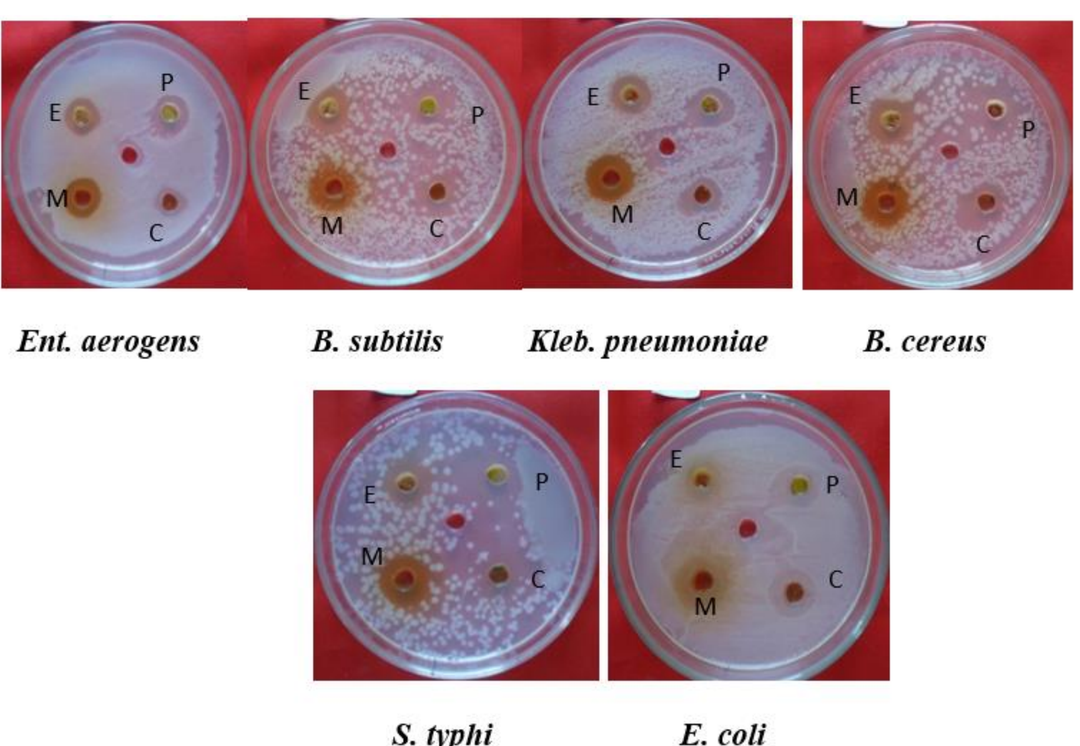

Figure 2. Antibacterial activity of different solvent extracts of *A. graveolens* against human pathogenic test bacteria by Cup diffusion method. Extracts of: P- Petroleum ether, C- Chloroform, E- Ethyl acetate, M-Methanol.

3.2. Antioxidant activity of A. graveolens.

DPPH radical scavenging assay of *A. graveolens* revealed that all the solvent extracts recorded a better scavenging ability than the standard ascorbic acid. The methanol extract of the plant recorded least value of 19 µg/ ml followed by ethyl acetate (21 µg/ ml), petroleum ether (24 µg/ ml) and chloroform (25 µg/ ml) extracts. All the extracts of the plant possessed a good scavenging ability when compared to the standard ascorbic acid. IC_{50} values of different extracts of *A. graveolens* by hydroxyl radical scavenging exhibited efficiency in the order of BHA > methanol > ethyl acetate > chloroform > petroleum ether (Table 5). The IC_{50} values showed that methanol extract was nearly potent to BHA. The efficacy of the extract in scavenging nitric oxide was good in methanol and ethyl acetate extracts expressed as IC_{50} values and compared with the standards.

Table 5. The IC_{50} values of DPPH radical, hydrogen peroxide, and nitric oxide radical scavenging by different extracts of *A. graveolens*.

Solvent extract	IC_{50} (µg/ ml)		
	DPPH	**H_2O_2**	**Nitric oxide**
Petroleum ether	24±0.33	34±0.72	48±0.31
Chloroform	25±0.12	38±0.55	41±0.41
Ethyl Acetate	21±0.55	32±0.32	40±0.24
Methanol	19±0.94	28±0.36	36±0.64
AA	25±0.34	---	23±0.21
BHA	---	22±0.08	---

Values are mean of three independent replicates ± Standard Deviation.

The antioxidant efficacy of all the extracts of *A. graveolens* by DPPH, hydrogen peroxide, and nitric oxide radical scavenging methods increases with the increase in concentration. The data are depicted in Figures 3, 4, and 5, respectively.

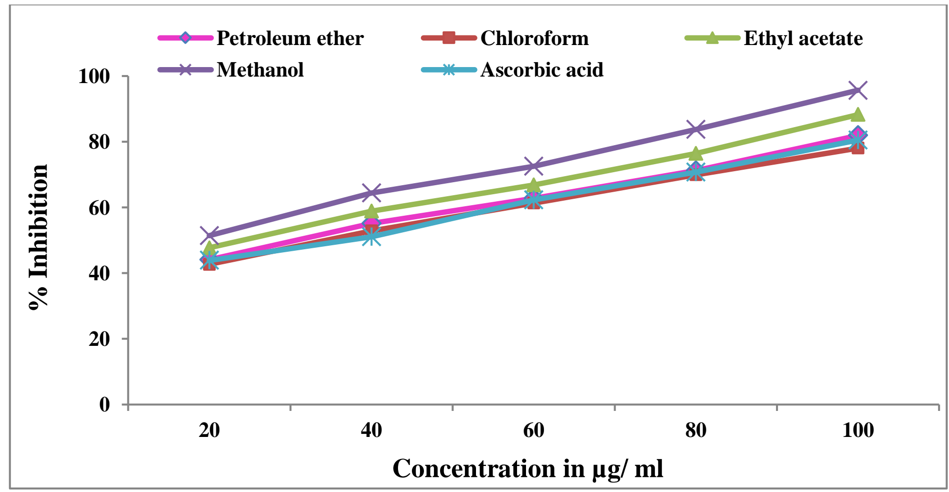

Figure 3. DPPH free radical scavenging activity of different extracts of *A. graveolens* and standard Ascorbic Acid at different concentrations.

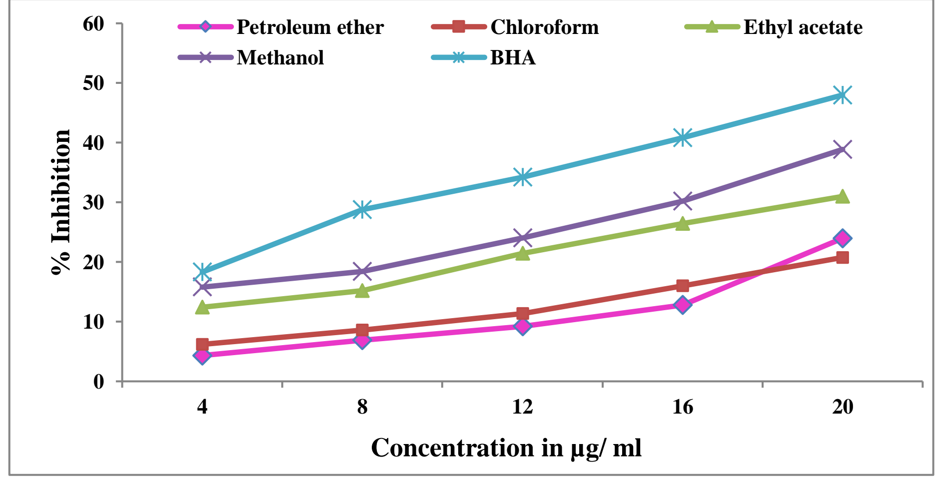

Figure 4. Hydroxyl radical scavenging activity of different extracts of *A. graveolens* and standard BHA at different concentrations.

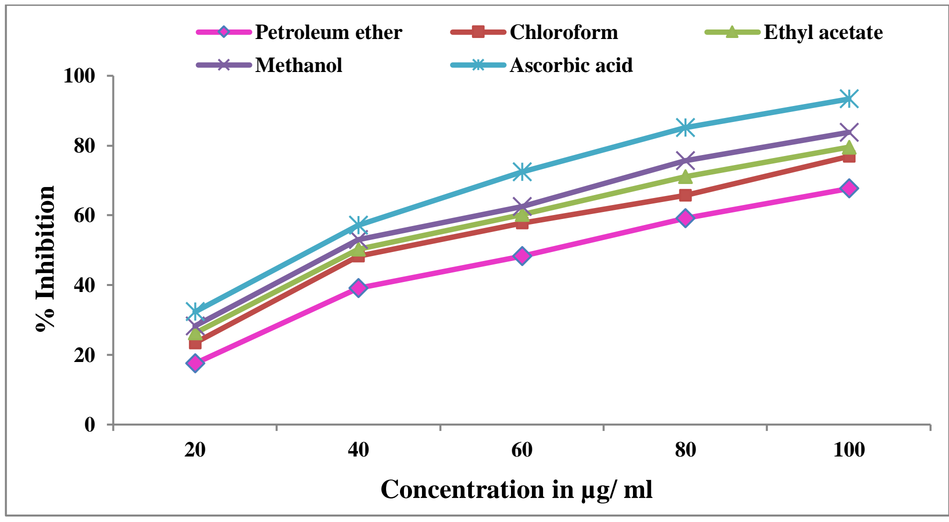

Figure 5. Scavenging effect of nitric oxide radical of different extracts of *A. graveolens* and standard AA at different concentrations.

3.3. Phytochemical analysis.

Phytochemical analysis of solvent extracts is presented in Table 6. Phenols, tannins, flavonoids, and proteins were present in methanol and ethyl acetate extracts. Methanol and petroleum ether extracts also contained steroids. Steriods and glycosides were present in all extracts.

Table 6. Phytochemical analysis of solvent extracts of *A. graveolens.*

Phytochemical compounds	Extracts			
	Petroleum ether	Chloroform	Ethyl acetate	Methanol
Alkaloids	-	-	-	-
Flavonoids	-	-	+	+
Terpenoids	-	-	+	+
Tannins	-	-	+	+
Steroids	+	+	+	+
Glycosides	+	+	+	+
Carbohydrates	+	+	-	-
Proteins	-	-	-	-
Saponins	-	-	-	-

+ = Present; - = Absent

4. Discussion

Many vital phytochemicals are obtained from various parts of the plants. Herbal remedies in traditional folk medicine are found to be a suitable alternative for the development of active new drugs for chemotherapy, which could overcome the growing problems of drug resistance and avoid the effects of the currently available antibiotics. The increasing interest in secondary metabolites and their medicinal properties has prompted to search for new compounds. Thus, the proposed work describes the cited considerations and the antibacterial and antioxidant activity of different solvent extracts of *A. graveolens.*

Remarkable antibacterial activities were obtained from all the extracts of *A. graveolens* against human pathogenic bacteria and good activity of 18-19 mm inhibition zone with methanol extract. The review found that the antibacterial activity of *A. graveolens* against human pathogenic bacteria from its essential oil, while crude soxhlet extracts activity was insignificant. But in the present study, methanol extract obtained by soxhlet extraction has

recorded promising activity. Many researchers have demonstrated that the essential oil of *A. graveolens* showed a significant antibacterial activity [22-25]. The literature survey suggests that highly polar solvents such as acetone, ethanol, and methanol extracts possess potential activity, which has also been evident from the present investigation [26,27]. This suggests that most of the active compounds are extracted better by high polar solvents.

The IC_{50} values of *A. graveolens* showed that methanol extract was nearly potent than the standard in all three methods. All the extracts of the plant possessed a good scavenging ability when compared to the standard ascorbic acid. Antioxidant capacity of *A. graveolens* was studied by earlier workers with infusions and decoctions [27], *Anethum* oil [26], alcohol extracts [28,29], but most of the studies were on the essential oils, while in the present research, different soxhlet extracts of *A. graveolens* were subjected to antioxidant assay, and methanol extract followed by ethyl acetate and chloroform extracts were found to be potent antioxidants. The choice of solvent for extraction, maturity of the samples, and extraction techniques may have a profound effect on observed antioxidant content and capacity, resulting in variation in the scavenging efficacy of the extracts[30].

The antioxidant activity comprises free radical scavenging capacity, reducing ability, metal ion chelating ability, and inhibition of lipid peroxidation. DPPH was used as a substrate to evaluate the free radical scavenging activity of *A. graveolens* extracts. DPPH radical contains an odd electron responsible for the formation of the blue color [31]. When DPPH accepts an electron from the extract, it gets converted into colorless diphenyl-picryl hydrazine. The reduced DPPH could be quantified spectrophotometrically at 517 nm. The methanol extract of the plant recorded the least value of 19 µg/ ml followed by ethyl acetate (21 µg/ ml), petroleum ether (24 µg/ ml), and chloroform (25 µg/ ml) extracts, indicating that all extracts have better scavenging activity as compared to ascorbic acid.

Hydrogen peroxide is a well-known oxidizing agent but, on few occasions be toxic to the host cell due to the hydroxyl radical. By oxidation of these thiol groups (-SH) it can inactivate few enzymes directly. The oxidation of (-SH) groups by oxidants may thus lead to disruption of various cellular functions and even cell death [32]. The extracts have shown H_2O_2 decomposing activity at a lower concentration of 28, 32, 34, 38, 28µg /ml of methanol, E. acetate, petroleum ether, and chloroform extracts, respectively slightly lesser than the standard BHA.

Nitric oxide is a potent pleiotropic mediator of the physiological process such as smooth muscle relaxant, neuro-signaling, platelet aggregation inhibition, and cell-mediated toxicity regulation. The NO reacts with superoxide and gives rise to various other reactive nitrogen species (RNS) such as NO_2, N_2O_4, and peroxynitrite, which attack and damage various cellular molecules[33]. Methanol extract showed scavenging activity at a concentration slightly higher than that of the standard by decreasing the amount of nitrite, which is toxic to the cell.

Similar results were observed in the antioxidant capacity of chloroform extract of *A. graveolens* where the extract recorded low phenol content and better antioxidant capacity when compared to other extracts. The correlation between total phenol content and antioxidant activity can be influenced by extraction procedures, assay methods, solvent, and variation in the nature of compounds. The antioxidant capacity of *A. graveolens* was studied by earlier workers with infusions and decoctions[26,27] *Anethum* oil, alcohol extracts [28,29]. However, most of the studies were on essential oils. Nonetheless, in the present research, different soxhlet extracts of *A. graveolens* were subjected to antioxidant assay. Methanol extract followed by ethyl acetate and chloroform extracts was found to possess potent antioxidant properties. The

choice of solvent for extraction, maturity of the samples, and extraction techniques may have a profound effect on observed antioxidant content and capacity, which result in variation in the scavenging efficacy of the extracts[30]. The chemical complexity of extracts, often a mixture of compounds with different functional groups, polarity, and chemical behavior, could lead to scattered results depending on the test employed.

There are several methods for the determination of antioxidant activities. The chemical complexity of extracts, often a mixture of compounds with different functional groups, polarity, and chemical behavior, could lead to scattered results depending on the test employed. Therefore, an approach with multiple assays for evaluating the antioxidant potential is appropriate[34].

The review on antimicrobial activity suggests that activity is more in organic solvent extracts because the antimicrobial principle was either polar or non-polar. In the sequential extraction technique, the chemical constituents are partially separated according to their polarity, the least polar components extracted into low polar solvents and high polar components extracted into the higher polar solvents, which leads to progressive separations of the active compounds. This partial separation of active components may be an advantage to reduce the antagonistic effects of chemical constituents present in crude mixture may interfere with the action of the other[35]. This has been evident in the present investigation. The selected plant material was sequentially extracted in the current study with different solvents to increase polarity, which plays an important role in the interaction of natural products, which influences the antibacterial activity.

The study on crude extracts is advantageous to additive or synergetic effects of the mixture, resulting in an increased antimicrobial spectrum of the extract and decreased risk of pathogen resistance to a mixture of active compounds[36]. The activity of all the extracts of selected plants was tested by employing both agar cup and disc diffusion methods. The inhibition zone was higher in the cup diffusion method in all the studied plant extracts when compared with the disc diffusion method, which may be due to the maximum diffusion of the extracts into the media, leading to the spread of the phytochemical or active compounds of the extracts to a wider diameter resulting in higher inhibition zone [37].

5. Conclusions

In conclusion, plant extracts rich in secondary metabolites are quite safe, and their toxicity is not a problem of concern, unlike those of BHA and synthetic drugs. Methanol extracts of *A. graveolens* possess a broad spectrum of activity against several human pathogenic and potent antioxidant properties. A natural substance obtained from plants, a part of a daily diet being a nutritional supplement with antimicrobial and antioxidant properties, constitutes a new source of herbal drugs.

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Conflicts of Interest

The authors declare no conflict of interest.

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