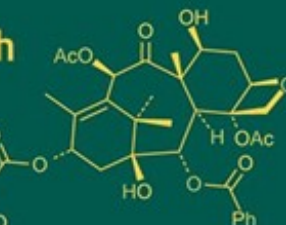


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
Indexing: Embase
IJABR 2026; SP-10(9): 2427-2432
www.biochemjournal.com
Received: 09-07-2026
Accepted: 26-08-2026
Published: 26-09-2026

Sarita Jagannath Suryavanshi

Dr. John Barnabas Post
Graduate School for
Biological Studies, Dr. Bhaskar
Pandurang Hiwale Education
Society's Ahmednagar
College, Ahilyanagar,
Maharashtra, India

Akash Baban Gore

Dr. John Barnabas Post
Graduate School for
Biological Studies, Dr. Bhaskar
Pandurang Hiwale Education
Society's Ahmednagar
College, Ahilyanagar,
Maharashtra, India

Phytochemical analysis, antioxidant and antimicrobial activities of *Anethum graveolens* (dill) leaf extracts

Sarita Jagannath Suryavanshi¹, Akash Baban Gore²

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i9Sad.10193>

ABSTRACT

Anethum graveolens (dill) leaf extracts were prepared using water, ethanol, and acetone and subjected to phytochemical analysis. Flavonoids, phenolics, coumarins, and terpenoids were detected in all three extracts. Thin-layer chromatography (TLC) indicated presence of coumarins. Phenolic and flavonoid contents were estimated from all three extracts where water extract had higher phenolic content; while acetone extract had more flavonoid content. The reducing power, antioxidant ability using phosphomolybdenum and 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ability to inhibit lipid peroxidation were assessed where water extract was found to be highly efficient than other two extracts. When the antimicrobial activity was investigated only acetone extract showed activity against the fungus *Aspergillus niger*. No inhibitory effect of any extracts was observed against the tested bacteria *E. coli*, *B. subtilis*, and *S. aureus* under the experimental conditions.

Keywords: *Anethum graveolens*, Antimicrobial activity, DPPH, Lipid peroxidation

1. Introduction

Free radicals and reactive oxygen species (ROS) produced during different biological processes can damage DNA, proteins, and lipids. This can lead to different types of diseases and disorders such as diabetes, cancer, cardiovascular diseases, immunosuppression, and neurological problems (Bahramikia *et al.*, 2008; Okorie *et al.*, 2019) [1, 2]. Defence mechanisms are present in the system but they are not sufficient to handle higher levels of ROS. Hence administration of antioxidants is required. Synthetic antioxidants cannot be given for long term due to their adverse effects on human health. Dietary plants are rich in antioxidants like flavonoids, carotenoids, terpenoids, phenolic acids, ascorbic acid, etc. One such plant is *Anethum graveolens* L., commonly called as dill, is a seasonal herb from the Apiaceae family. A number of different compounds have been obtained from dill namely phenolic acids, α -phellandrene, triterpenes, flavonoids, coumarins, etc (Jana *et al.*, 2010) [3]. Dill is widely cultivated throughout the world. It is used to savour various foods, including salads, sauces, soups, pickles, and seafood (Sebnem *et al.*, 2011) [4]. Its seeds are used as spice. Dill also has medicinal roles as diuretic, anti-convulsion, wound-healer and used against gastrointestinal problems, including flatulence, intestinal spasms, etc (Sebnem *et al.*, 2011; Nguyen *et al.*, 2020; Wasli *et al.*, 2018) [4, 5, 6]. It also shows anti-hyperlipidaemic and anti-hypercholesterolemic activities (Bahramikia *et al.*, 2008; Oshaghi *et al.*, 2017) [1, 7]. Oil obtained from different parts of dill has antioxidant and antimicrobial properties. Many investigators have worked on dill seeds or oil from dill seeds but few have worked on dill leaves. Dill is a common vegetable, abundantly available in market. Hence in the present work, dill leaves were used to study their antioxidant and antimicrobial activities. Extracts of dill leaves were prepared in water, ethanol, and acetone, and subjected to phytochemical analysis. The phenolic and flavonoid contents were estimated. The reducing power, antioxidant ability using phosphomolybdenum and DPPH were determined. Free radicals are responsible for lipid peroxidation that also results in different diseases and aging. This damage can be prevented by antioxidants. Hence, in the present study, the ability of dill leaf extracts to inhibit lipid peroxidation was also investigated.

2. Materials and Methods

2.1. Chemicals

The solvents and reagents used in this research were of analytical grade and from SD Fine chemicals, Loba chemie, Qualigens, etc.

2.2. Collection of plant material and preparation of sample

Dill vegetable was purchased from a local market. Its leaves were removed and dried in the shade. The dried leaves were divided into three parts (7 g each) and subjected to solvent extraction using water, ethanol and acetone, respectively, in a Soxhlet apparatus. The collected extracts were stored at 4 °C until further analysis.

Corresponding Author:

Sarita Jagannath Suryavanshi

Dr. John Barnabas Post
Graduate School for
Biological Studies, Dr.
Bhaskar Pandurang Hiwale
Education Society's
Ahmednagar College,
Ahilyanagar, Maharashtra,
India

Different tests were performed on all three dill leaf extracts (henceforth referred to as sample).

2.3. Phytochemical screening

The screening of phytochemicals such as flavonoids, phenolics, reducing sugars, saponins, steroids, etc in all three samples was done using standard protocols (Anyasor *et al.*, 2010; Mehta *et al.*, 2017; Kaur *et al.*, 2018) [8, 9, 10] as mentioned in Table 1.

2.4. TLC

The samples were applied to pre-coated TLC plates using glass capillaries. TLC was performed using solvent Toluene-Ether (1:1) saturated with 10% acetic acid (Kovac Besovic *et al.*, 2003) [11]. After drying, the plates were exposed to UV light (254 nm). The spots were observed, and the R_f values were calculated.

2.5. Determination of phenolic content

The phenolic content of the samples was determined using the Folin-Ciocalteu (FC) reagent (Sebnem *et al.*, 2011; Singleton *et al.*, 1999) [4, 12]. One ml of suitably diluted sample was taken, and 1 ml of FC reagent was added to it. The mixture was allowed to stand at room temperature for 5 min, then 2.5 ml of sodium carbonate (2%, w/v) was added. The tubes were kept at room temperature for 30 min. The blue color developed was measured at 720 nm. Phenolic content was determined using gallic acid as standard.

2.6. Estimation of flavonoids

The flavonoid content was estimated according to the method described by (Singh and Chahal) [13], with slight modification. To one ml of suitably diluted sample, 0.05 ml of 10% aluminium chloride and 0.05 ml of 1 M potassium acetate were added. The total volume was adjusted to 5 ml with 80% methanol. Absorbance was measured at 420 nm. Quercetin was used as standard.

2.7. Reducing power assay

The reducing power of the sample was determined according to the method reported by (Sebnem and Ayten) [4]. The diluted samples (1 ml) were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and

2.5 ml of potassium ferricyanide (1%), and incubated at 50 °C for 20 min. After incubation, 2.5 ml of trichloroacetic acid (TCA) (10%) was added, and the mixture was centrifuged at 3000 rpm for 10 min. The upper layer solution was mixed with distilled water and FeCl_3 (0.1%), and the absorbance was measured at 700 nm and compared with that of ascorbic acid.

2.8. Antioxidant activity by phosphomolybdenum method

The antioxidant activity of the samples was evaluated using the phosphomolybdenum method (Prieto *et al.*, 1999) [14]. It is based on the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of a phosphate/Mo (V) complex at acidic pH. One ml of each sample with suitable dilution was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate). The mixture was covered with aluminium foil and incubated at 95 °C in a thermal block for 90 min. After incubation, the mixture was cooled to room temperature, and the absorbance was measured at 695 nm. Ascorbic acid was used as standard. The antioxidant activity was expressed as μg ascorbic acid equivalents.

2.9. Determination of radical scavenging activity using DPPH

The radical scavenging activity of sample was investigated based on the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical to a corresponding hydrazine on reacting with hydrogen donors. The violet color of the DPPH radical turns to yellow on reduction.

In the present study, 0.1 ml of sample was taken, and the total volume was made to 1 ml with ethanol. To all the tubes, 2 ml of 0.1mM DPPH solution prepared in ethanol was added. The negative control contained 1 ml ethanol and 2ml DPPH solution. A positive control was also prepared using the antioxidant, ascorbic acid. All tubes were kept in the dark at room temperature for 30 min. Absorbance was measured at 517 nm. DPPH scavenging effect was calculated using the equation: (Wasli *et al.*, 2018) [6]

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

where the absorbance of control is the absorbance without extract (negative control) and absorbance of sample is the absorbance with extract.

The effect of increasing the volume of the sample on DPPH scavenging activity was checked and compared with that of ascorbic acid (10 $\mu\text{g}/\text{ml}$). The volumes used were in the range of 0.05 ml to 0.35 ml. The total volume was adjusted to 1 ml with ethanol. The same procedure was followed as mentioned above.

2.10. Thiobarbituric acid reactive species (TBARS) assay to determine lipid peroxidation

In the present study, a modified TBARS assay was used to measure the formation of lipid peroxide using egg yolk homogenate as a lipid-rich medium, as reported by (Dissanayake *et al.*) [15]. Egg yolk contains unsaturated fatty acids. These unsaturated fatty acids when react with oxygen, produce malondialdehyde (MDA). The MDA then re-

acts with thiobarbituric acid (TBA) to give orange-red color, which is measured at 532 nm (Ohkawa *et al.*, 1979) [16]. The antioxidants, if present in the sample, will reduce the formation of MDA thus resulting in decrease in color with TBA. In the present study, egg yolk was collected, and a 10% (v/v) solution was prepared in 1.15% KCl and homogenized. TBA 0.8% (w/v) was prepared in 1% sodium dodecyl sulphate (SDS). The egg yolk homogenate (0.5 ml) was added to each sample (0.1 ml). The volume was then adjusted to 1.0 ml with distilled water. Then, 0.05 ml of FeSO_4 was added and the mixture was incubated at 37°C for 30 min. Subsequently, 1.5 ml of acetic acid was added, followed by 1.5 ml of TBA in SDS. The resulting mixture was vortex-mixed and heated at 95°C for 1 h. After cooling, 5 ml of butanol was added, and the mixture was centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm, and the percentage inhibition was calculated using the following formula:

$$\% \text{ inhibition of lipid peroxidation} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

where Absorbance of control = Absorbance of fully oxidized control and

Absorbance of sample = Absorbance of test sample

The same procedure was performed for the positive control, ascorbic acid.

Effect of increasing volume of the sample (0.05 ml to 0.2 ml) was also checked on lipid peroxidation.

2.11. Antimicrobial activity

The antimicrobial activity of all three samples was checked. The test organisms used were *E. coli*, *S. aureus*, *B. subtilis* and *Aspergillus niger* obtained from Microbiology Department of Ahmednagar College. Nutrient agar medium was used for bacteria and potato dextrose agar (PDA) for fungi. The bacteria were spread on solidified nutrient agar plates. Sterile discs, sized at 6 mm, were loaded with 15 μl

of the extract and placed onto the bacterial culture plates. Plates without discs served as controls. Incubation was carried out for 24 h at 37 °C. (Sonawane *et al.*, 2025; Behbahani *et al.*, 2025) [17, 18]. To check antifungal activity agar well diffusion method was used. Petri plates were prepared by pouring 30 ml of PDA medium. The surfaces of medium were inoculated with fungus *Aspergillus niger*. Wells were made using sterile cork-borer and 15µl of each sample was dispensed in respective wells. Solvent without extract served as negative control. The plates were incubated for 48h at 37 °C.

3. Results and Discussion

3.1. Phytochemical analysis

The results of phytochemical analysis are given in Table 2 and in Fig 1 and 2.

3.2. TLC

When the samples were subjected to TLC, migration was observed only of acetone extract with R_f value 0.7 cm (Fig.3). It indicates presence of coumarins (Kovac Besovic *et al.*, 2003) [11].

3.3. Estimation of phenolic and flavonoid contents

The phenolic and flavonoid contents from all three samples were determined and expressed in terms of gallic acid and quercetin equivalents, respectively. The phenolic content was more in water extract then ethanol followed by acetone extracts (Fig. 4); while the reverse order was noticed for flavonoid content (Fig. 5, Table 3).

Literature data showed mixed results. (Bahramikia and Yazdanparast) [1] reported highest phenolic and flavonoid contents in ethyl acetate fraction and lowest in water fraction of dill leaf. (Sebnem and Ayten) [4] reported higher phenolic content in water and ethanol extracts than in acetone extract. (Nguyen *et al.*) [5] reported highest phenolic and flavonoid content in ethanol extract than in water; while (Jadhav and Pawar) [19] reported high content of phenolics and flavonoids in acetone extract as compared to other solvent extracts.

3.4. Reducing power assay and determination of antioxidant activity

The reducing power and antioxidant activity using phosphomolybdenum method of sample were determined and expressed in terms of ascorbic acid equivalents. In both cases higher activity was noticed for water extract and least for acetone extract (Table 3).

(Sebnem and Ayten) [4] reported higher reducing power in water and ethanol extracts than in acetone extract.

3.5. DPPH assay

Water extract of dill leaf showed higher DPPH radical scavenging activity as compared to ethanol and acetone extracts. Therefore, water extract was used further to find out effect of increasing volume on DPPH radical scavenging activity. Increase in free radical scavenging activity was seen with increase in volume (from 0.05 ml to 0.35 ml) where 0.35 ml of dill leaf extract resulted in 85% scavenging activity; while 0.35 ml of ascorbic acid (3.5µg concentration) resulted in 65% scavenging activity (Fig.6).

(Bahramikia and Yazdanparast) [1] reported higher scavenging activity of ethyl acetate fraction of dill leaf as compared to diethyl ether and water fractions; while (Nguyen *et al.*) [5] reported for methanol extract as against aqueous extract. According to (Sebnem and Ayten) [4] water and ethanol extracts showed similar DPPH radical scavenging activity; while acetone extract was less effective.

3.6. Inhibition of lipid peroxidation

Inhibition of lipid peroxidation was maximum due to water extract than ethanol extract; while least effect was observed of acetone extract. Hence effect of increase in volume of water extract (from 0.05 ml to 0.2 ml) was checked on lipid peroxidation and compared with that of ascorbic acid where 0.2 ml of extract resulted in 65% inhibi-

tion (Fig. 7), same volume of ascorbic acid (with 0.2 mg concentration) resulted in only 31% inhibition.

(Bahramikia and Yazdanparast) [1] reported higher inhibition of lipid peroxidation by ethyl acetate fraction than other solvent fractions.

3.7. Antimicrobial activity

Neither of the three extracts was effective against the tested bacteria *E. coli*, *S. aureus*, and *B. subtilis* under the experimental conditions. The activity of all the three extracts when checked against the fungus *Aspergillus niger*; only acetone extract was found to be effective. Pure acetone was used as negative control. (Rasheed *et al.*) [20] reported no effect of dill leaf and stem extract on the growth of *S. aureus*, *E. coli*, and *C. albicans*; but of seed oil on *C. albicans*. (Jana and Shekhawat) [21] reported in vitro antimicrobial activity of ethanolic extract of dill leaves against *E. coli*, *B. cereus*, *B. subtilis*, and *M. luteus*.

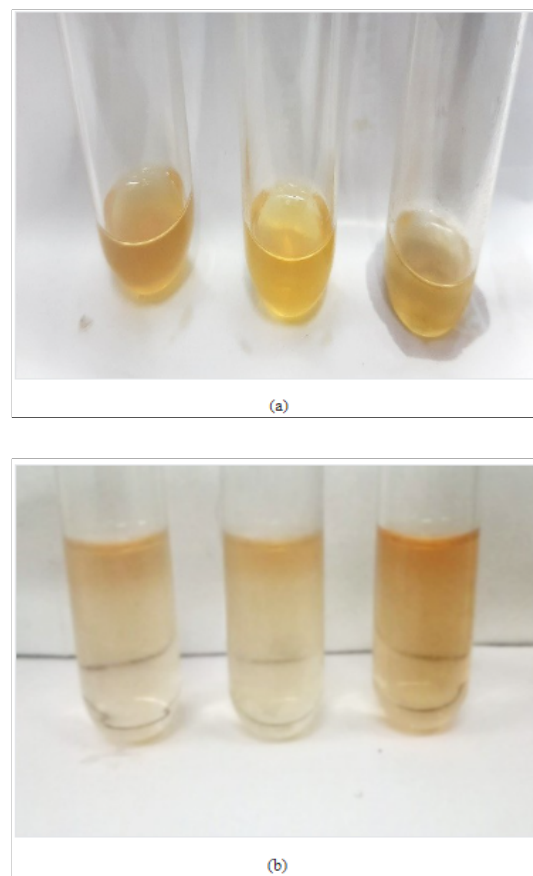
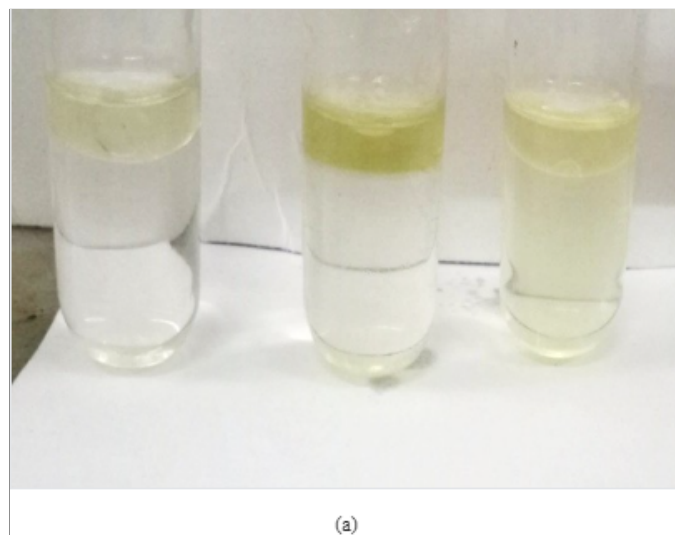


Figure 1. Tests for a) Coumarin and b) Cardiac glycosides (Dill leaf extracts: Water, Ethanol, Acetone, respectively)



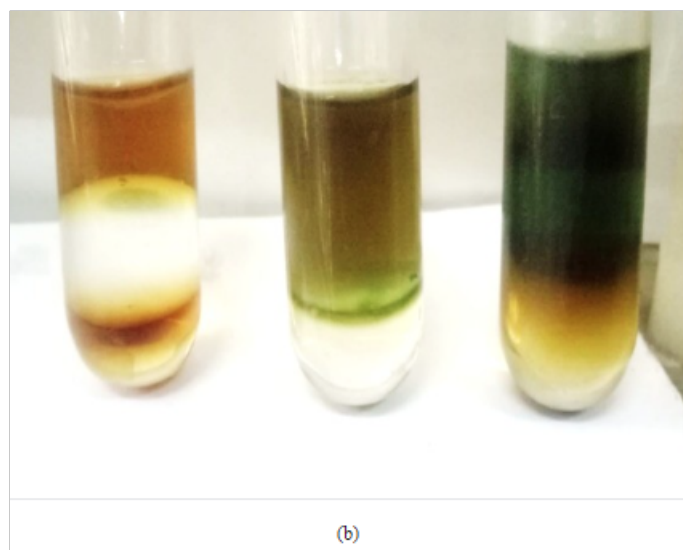


Figure 2. Tests for a) Saponins and b) Terpenoids (Dill leaf extracts: Water, Acetone, Ethanol, respectively)

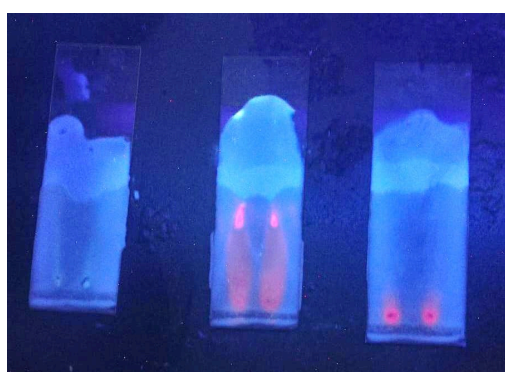


Figure 3. TLC of dill leaf extracts in water, acetone, and ethanol, respectively

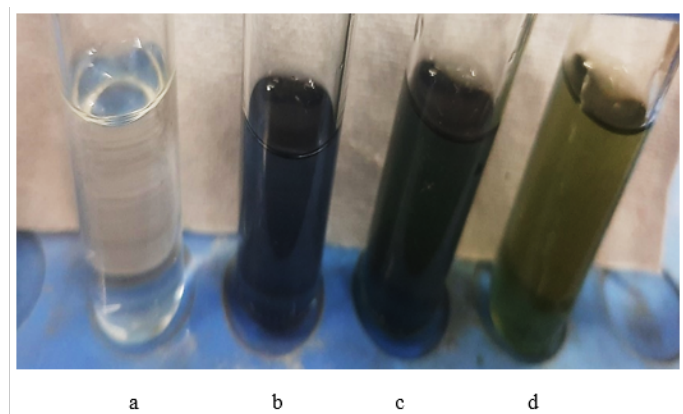


Figure 4. Phenolic content of dill leaf extracts

a: Blank, b: water extract, c: ethanol extract, d: acetone extract

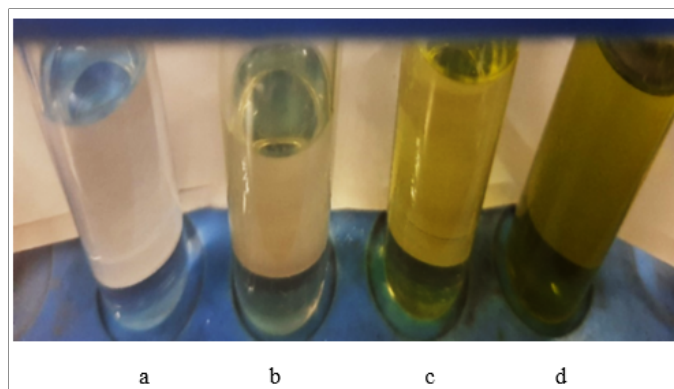


Figure 5. Flavonoid content of dill leaf extracts

a: blank, b: water extract, c: ethanol extract, d: acetone extract

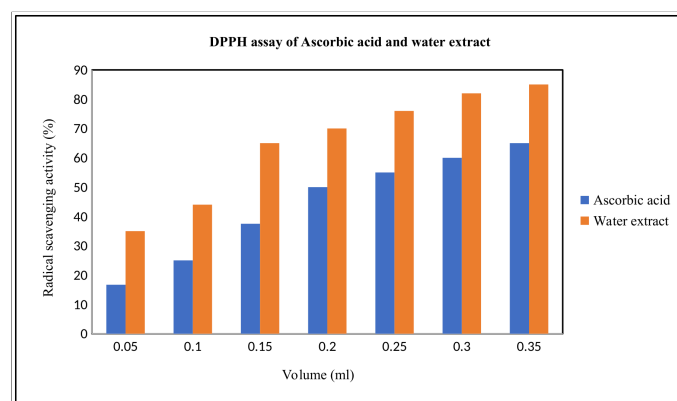


Figure 6. Effect of increase in volume of ascorbic acid and water extract of dill leaf on DPPH scavenging ability

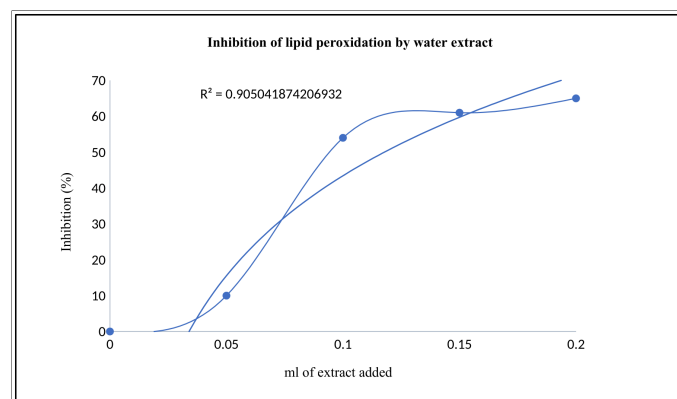


Figure 7. Effect of increase in volume of water extract of dill leaf on inhibition of lipid peroxidation

Table 1. Phytochemical screening protocols

Phytochemicals	Procedure	Observation
Flavonoids	1 ml extract + 1% of aluminium chloride	Intense yellow colour
Phenolics	1 ml extract in water + few drops of 5% ferric chloride	Dark green colour
Coumarin	Extract+ alcoholic FeCl ₃ + Con. HNO ₃	Green Yellow
Reducing sugars	1 ml extract + Fehling's solution A and B in equal volume	Red precipitate
Saponins	1 ml of extract boiled with 10 ml distilled water, shaken vigorously, 2-3 drops of olive oil	Emulsion
Anthraquinones	1 ml extract boiled with 1% hydrochloric acid + 3 ml benzene + 2 ml 10% ammonia solution	Pink, violet or red colour

(continued on next page)

Cardiac glycoside	1 ml extract + 2 ml glacial acetic acid + few drops of ferric chloride solution	Brown ring at interface
Terpenoids	1 ml extract + 2 ml chloroform +3 ml sulfuric acid	Reddish brown colour at interface
Phlobatannins	1 ml extract was boiled with few drops of 1% hydrochloric acid	Red precipitate

Table 2. Results of phytochemical analysis from dill leaf extracts

Phytochemicals	Acetone extract	Ethanol extract	Water extract
Flavonoids	+	+	+
Phenolics	+	+	+
Coumarins	+	+	+
Reducing sugars	-	-	-
Saponins	+	+	+
Anthraquinones	+	+	-
Cardiac glycoside	+	+	+
Terpenoids	+	+	+
Phlobatannins	-	-	-

Table 3. Phenolic and flavonoid contents, reducing power and antioxidant activity of dill leaf extracts

Dill leaf extract	Phenolic content (µg/ml) ^a	Flavonoid content (µg/ml) ^b	Reducing power (µg/ml) ^c	Antioxidant activity (µg/ml) ^c
Water	1050	500	600	1020
Ethanol	950	510	250	600
Acetone	800	640	150	390

Footnote:

a = expressed as µg gallic acid equivalents/ml of extract,

b = expressed as µg quercetin equivalents/ml of extract, and

c = expressed as µg ascorbic acid equivalents/ml of extract

4. Conclusion

Based on the experimental results, it can be concluded that *A. graveolens* leaves are rich in antioxidants which are highly efficient in free radical scavenging and inhibition of lipid peroxidation in the Lab. Inclusion of dill herb in the diet therefore can protect people from different diseases caused due to free radicals and ROS. The dill herb extracts may be used in phytochemical drugs and in food industry.

5. Conflict of interest

No conflict of interest to declare.

References

- Bahramikia S, Yazdanparast R. Antioxidant and free radical scavenging activities of different fractions of *Anethum graveolens* leaves using in vitro models. *Pharmacologyonline*. 2008;2:219-233.
- Okorie NH, Mbah CJ, Orabueze I. Antioxidant Properties of Natural and Synthetic Chemical Compounds: Therapeutic Effects on Biological System. *Acta Sci Pharm Sci*. 2019;3(6):28-42.
- Jana S, Shekhawat GS. *Anethum graveolens*: An Indian traditional medicinal herb and spice. *Pharmacognosy Reviews*. 2010;4(8):179-184.
- Sebnem SI, Ayten S. Antioxidant Potential of Different Dill (*Anethum Graveolens* L.) Leaf Extracts. *Int J Food Prop*. 2011;14(4):894-902.
- Nguyen VT, Nguyen NQ, AN TNT, Van NT, Anh NHT. Evaluation of polyphenol content and antioxidant activities of Dill leaves extract *Anethum Graveolens* L. *IOP Conf Ser.: Mater Sci Eng*. 2020;991:012032.
- Wasli H, Jelali N, Ellouzi H, Msaada K, Silva AMS, Cardoso SM, et al. Enzymatic and non-enzymatic antioxidant activities of dill (*Anethum graveolens* L.) in response to bicarbonate-induced oxidative damage. *Agrochimica*. 2018;LXII(4):319-335.
- Oshaghi EA, Khodadadi I, Mirzaei F, Khazaei M, Tavilani H, Goodarzi MT. Methanolic extract of dill leaves inhibits AGEs formation and shows potential hepatoprotective effects in CCl₄ induced liver toxicity in rat. *J Pharm (Cairo)*. 2017;6081374.
- Anyasor GN, Ogunwenmo KO, Oyelana OA, Akpofunre BE. Phytochemical constituents and antioxidant activities of aqueous and methanol stem extracts of *Costus afer* Ker Gawl. (Costaceae). *Afr J Biotechnol*. 2010;9(31):4880-4884.
- Mehta S, Rana P, Saklani P. Phytochemical Screening and TLC Profiling of Various Extracts of *Reinwardtia indica*. *Int J Pharmacogn Phytochem Res*. 2017;9(4):523-527.
- Kaur N, Chahal KK, Singh R, Urvashi. Phytochemical screening and antioxidant activity of *Anethum graveolens* L. seed extracts. *Pharma Innov J*. 2018;7(6):324-329.
- Kovac Besovic EE, Duric K. Thin layer chromatography-application in qualitative analysis on presence of coumarins and flavonoids in plant material. *Bosn J Basic Med Sci*. 2003;III(3):19-26.
- Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu Reagent. *Methods Enzymol*. 1999;299:152-178.
- Singh R, Chahal KK. Phytochemical analysis and in vitro antioxidant capacity of different solvent extracts of *Saussurea lappa* L. roots. *J Pharmacogn Phytochem*. 2018;7(3):427-432.
- Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Anal Biochem*. 1999;269(2):337-341.
- Dissanayake DP, Abeytunga DTU, Vasudewa NS, Ratnasooriya WD. Inhibition of lipid peroxidation by extracts of *Pleurotus ostreatus*. *Pharmacogn Mag*. 2009;4(19):266-271.

16. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.* 1979;95:351-358.
17. Sonawane KB, Rahangdale SB, Suryavanshi SJ. Extraction, purification, and antimicrobial activity of a pigment from *Aspergillus* species isolated from soil. *Int J Sci Res Arch.* 2025;15(3):118–123.
18. Behbahani BA, Golbashy M. Evaluation of the antimicrobial activity of dill aqueous extract and its interaction with chloramphenicol antibiotic: An in vitro study. *J Food Sci Tech No.* 161. 2025;22.
19. Jadhav VK, Pawar SS. Qualitative and quantitative analysis of *Anethum graveolens* L. leaves in various extracts. *Adv Zool Bot.* 2023;11(5):392-398.
20. Rasheed EM, Hamudi M, Kreem MR. Antimicrobial activity and the median lethal dose of dill (*Anethum Graveolens*) extract. *DASJ.* 2010;2(1):16–27.
21. Jana S, Shekhawat GS. Phytochemical analysis and antibacterial screening of in vivo and in vitro extracts of Indian Medicinal Herb: *Anethum graveolens*. *Res J Med Plant.* 2010;4(4):206-212.

How to cite this article: Suryavanshi SJ, Gore AB. Phytochemical analysis, antioxidant and antimicrobial activities of *Anethum graveolens* (dill) leaf extracts. *International Journal of Advanced Biochemistry Research.* 2026;SP-10(9):2427-2432. doi: <https://www.doi.org/10.33545/26174693.2026.v10.i9Sad.10193>