# Antioxidant and antibacterial properties of anise (*Pimpinella anisum* L.)

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**Background**
There is a great interest in the discovery of novel natural bioactive compounds in today's world. Plants, in particular, from different ecological niches and taxonomic groups are known to produce a high number of naturally occurring secondary metabolites, many of them with unique pharmacological activities. Therefore, the primary aim of the study was to investigate the antioxidant and antimicrobial activities of anise.
**Materials and methods**
The antioxidant and antimicrobial properties of different solvent extracts (ethanol and water) prepared from two parts (seeds and aerial parts) of *Pimpinella anisum* were evaluated. The antioxidant capacity was studied through the evaluation of the free radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl radical. The antimicrobial activity was analyzed using the well diffusion method, where zones of inhibition were used as indicators of antimicrobial activity.
**Results and conclusion**
The highest percentage of radical-scavenging activity (91.3±1.8%) was recorded for the ethanolic extract of seeds at a concentration of 0.3 mg/ml, followed by the aqueous extract of seeds (82.0±1.2%), whereas the aqueous extract of aerial parts demonstrated the lowest frequency of radical-scavenging activity (39.0±1.7%) at the same tested concentration. The largest inhibition zones were determined to be 21.0±1.2, 18.3±1.5, 9.7±1.2, and 7.0±1.2 mm for *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Escherichia coli*, respectively. On the whole, the results demonstrated the superiority of seed extracts over aerial part extracts. The results also indicated the stronger activity of ethanolic extracts compared with aqueous extracts. These results offer insights into the antioxidant and the antimicrobial potency of this Egyptian local plant and provide a basis for further phytochemical and pharmacological research.

**Keywords:**
agar well diffusion, antimicrobial activity, antioxidant property, 1, 1-diphenyl-2-picrylhydrazyl, *Pimpinella anisum*

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## Introduction

Free radical-induced oxidative damage plays a major part in the development of many chronic and degenerative ailments such as cancer, stroke, arthritis, autoimmune disorders, and cardiovascular diseases [1,2]. Oxidative stress which occurs when a biological system is incapable of keeping up with the detoxification of the free radicals is capable of damaging all kinds of molecules including important macromolecules such as nucleic acids, proteins, lipids, and carbohydrates [3]. The antioxidants neutralize the free radicals mainly through their free radical-scavenging property, thereby mitigating the effect of oxidative stress [4]. Although some antioxidants are produced naturally by the human body, the body cannot manufacture the principal micronutrient antioxidants such as vitamin E (α-tocopherol), vitamin C (ascorbic acid), and B-carotene [5]. Therefore, they must be supplied in the diet.

Infectious diseases are another major problem worldwide. Synthetic antibacterial drugs are not only expensive and inadequate but are also often with side effects. Moreover, because of the overuse and/or misuse of antibiotics through the past decades, many pathogens have evolved resistance to them via natural selection [6]. This problem is progressively becoming more and more intense in terms of frequency and severity. Nowadays, resistance is seen to nearly all antibiotics that have been developed [7].

The bioactive natural compounds represent a remarkable source of novel antioxidants and antimicrobial agents. These bioactive natural

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substances have shown reduced instances of side effects and good therapeutic potential [8]. Although a broad spectrum of organisms produces such bioactive compounds, the research to obtain these natural substances has been focused mainly on medicinal plants [9]. Plants are known to produce a high number of naturally occurring secondary metabolites, many of them with unique pharmacological activities. These metabolites include the flavonoids, phenolic acids, and tannins [10]. Owing to their versatile biological and pharmacological properties, food, nutrition, cosmetics, and pharmaceutical industries have found many applications for these compounds in the production of functional foods, nutritional composites, personal care products, and medicines [11–13]. Therefore, modern plant biotechnological tools were used to maximize the production of these secondary metabolites *via* plant cell cultures [14].

Anise (*Pimpinella anisum* L.) is one of the oldest known medicinal plants that belong to the family Apiaceae. It is an annual aromatic herb with 30–50 cm high, white flowers, and small green to yellow seeds, which grows in the Mediterranean area including Egypt, West Asia, and Europe [15]. Anise and its essential oil are globally used in food, medicine, perfumery, and cosmetic industries [16]. Despite the numerous precious uses of anise, there is a dearth of information in the literature on the biological activities of Egyptian anise. It is well known that the composition of anise and consequently its biological activities vary considerably with geographic origin and cultivation method. Hence the present study was conducted to evaluate the antioxidant and antibacterial activities of Egyptian local anise.

## Materials and methods

### Anise samples

Seeds of anise were obtained from the Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Egypt. The seeds were surface sterilized with 20% commercial Clorox (5% NaOCl) containing 0.1% Tween 20 for 20 min and thoroughly washed with sterilized, distilled water three times. Aseptic seeds were cultured on MS medium [17] without growth regulators and allowed to germinate under laboratory conditions. The medium was adjusted to pH 5.7 and supplemented with 0.7% agar and 3% sucrose. The cultures were incubated at 25±2°C under 16/8 h (light/dark) photoperiod. Aerial parts (harvested from 1-month-old seedlings) and seeds were used for the preparation of extracts.

### Preparation of extracts

Seeds and aerial parts were dried in oven at 40°C for 24 h. Afterwards, the two dried samples were ground into a fine powder. Ethanol and distilled water were used as solvents for the preparation of extracts. Ten grams of each of the two samples were soaked separately in 100 ml of each solvent and kept in a shaker for 2 days. The obtained mixtures were filtered through Whatman filter paper no.1. The filtrates were evaporated to dryness and the resulting viscous powders were dissolved in the same extract solvents to get a final concentration of 50 mg/ml stock solutions. These stock solutions of the four extracts were stored at 4°C until used.

### Antioxidant assay

The antioxidant capacity was studied through the evaluation of the free radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The determination was based on the method described by Brand-Williams *et al.* [18] with slight modification. Briefly, 500 µl of each extract (at different concentrations, that is, 0.05, 0.1, 0.2, and 0.3 mg/ml) was mixed with 2.5 ml of methanolic solution of DPPH (0.1 mmol/l). The mixture was kept in the dark for 30 min before the absorbance at 517 nm was measured against a control solution of methanol and DPPH without extracts. Ascorbic acid was used as positive control. The results were expressed as percentage of the DPPH radical. Radical-scavenging activity (RSC%) was calculated according to the following equation:

$$\mathrm{RSC}\,(\%) = \frac{A_{\mathrm{control}} - A_{\mathrm{sample}}}{A_{\mathrm{control}}} \times 100,$$

where $A_{\mathrm{control}}$ is the absorbance of DPPH without extract, while $A_{\mathrm{sample}}$ is the absorbance of the extracts.

### Microorganisms and culture media

The antimicrobial activities of anise extracts were determined against a panel of pathogens. Two Gram-negative bacteria (*Salmonella typhimurium* NCTC 12023 ATCC 14028; *Escherichia coli* ATCC 25922) and two Gram-positive bacteria (*Bacillus cereus* ATCC 33018; *Staphylococcus aureus* ATCC 25923) were provided by the Microbiological Resource Center, Faculty of Agriculture, Ain Shams University, Egypt. Bacterial strains were maintained on nutrient agar and stored at 4°C until use.

### Antimicrobial assay

Antimicrobial activity tests were conducted by using the agar well diffusion method [19]. Fifteen milliliters of the nutrient agar medium was added into petri

dishes. The melted and tempered (40°C) agar was previously inoculated with 200 µl of the target microorganism cell suspension. The freshly grown suspensions were prepared by diluting microbial cultures of the target strain to achieve a microbial concentration of $10^8$ CFU/ml. The agar plates were solidified for 1 h and then, using a sterile cylinder, wells of 8-mm diameter were made and filled up with 100 µl of the diluted stock solutions (plant extracts at a concentration of 1.25, 2.5, and 5 mg/ml). Wells containing solvents (100 µl) were used as a negative control, while wells containing tetracycline (a standard antimicrobial) served as a positive control (50 µg/ml). The plates were incubated for 48 h at 28°C. The antimicrobial activities of the anise extracts were evaluated by measuring the inhibition zones around the wells. The inhibition zones were measured with a ruler and were determined by a clear zone of at least 2 mm around the well.

### Statistical analysis

Statistical analysis was performed using IBM SPSS statistics subscription. One-way analysis of variance was used for statistical analysis. Five samples were used for each treatment and each experiment was repeated three times. Mean±SE were obtained from the analysis for each treatment. Data were presented as mean±SE and were compared with Tukey's test at a 5% probability level.

## Results

The antioxidant activities of the different solvent extracts, at various concentrations, prepared from various parts of *P. anisum* were investigated by the DPPH test system. The obtained results in Table 1 showed differences in the radical-scavenging activity percentage among the different types of extracts at the same concentrations. Also, the same extracts at different concentrations showed different radical-scavenging frequencies. In general, irrespective of the extract type, all samples exhibited considerable antioxidant activity at varying degrees. In this context, seed extracts proved to have higher ability to scavenge the DPPH radical than those of aerial parts. In addition, ethanol extracts were more potent than aqueous extracts. On a whole, the highest percentage of radical-scavenging activity (91.3±1.8%) was recorded for the ethanolic extract of seeds at a concentration of 0.3 mg/ml, followed by the aqueous extract of seeds (82.0±1.2%), whereas the aqueous extract of aerial parts demonstrated the lowest frequency of radical-scavenging activity at the same tested concentration (39.0±1.7%). It was observed that as the concentration of any anise extract increased, the antioxidant activity also increased. At high concentration (0.3 mg/ml), the antioxidant activities of both seed extracts (ethanolic and aqueous) were closer to the scavenging effect of ascorbic acid. In fact, statistical analysis indicated that there was no significant difference between the antioxidant activity of ethanolic extract of seeds (91.3±1.8%) and ascorbic acid (95.3±1.2%).

The antibacterial activities of anise extracts were tested against four pathogenic bacteria; the results of the tests on the two Gram-positive and two Gram-negative bacteria are presented in Table 2. The ethanolic extract of seeds showed antibacterial activity against all the species of bacteria investigated, with inhibition zones from 3.3±0.9 to 21.0±1.2 mm. The largest inhibition zones were observed against *B. cereus* and *S. aureus* (21.0±1.2 and 18.3±1.5 mm, respectively). The aqueous extract of seeds at a low concentration (1.25 mg/ml) inhibited the growth of only *S. aureus* and *B. cereus*, with inhibition zones of 2.7±0.3 and 3.3 ±0.7 mm, respectively. A higher concentration (5 mg/ml) extended this inhibition to include *S. typhimurium* and *E. coli* with inhibition zones of 4.0±0.6 and 2.7 ±0.7 mm, respectively. Solvent extracts of aerial parts were less effective as antimicrobial agents compared with solvent extracts prepared from the seeds of anise.

**Table 1 Antioxidant activity in anise extracts**

| | 1,1-Diphenyl-2-picrylhydrazyl inhibition (%) | | | | |
|---|---|---|---|---|---|
| Extract concentration (mg/ml) | Ethanolic extract of seeds | Aqueous extract of seeds | Ethanolic extract of aerial parts | Aqueous extract of aerial parts | Ascorbic acid |
| 0.05 | $56.3\pm1.5^{A,d}$ | $44.3\pm1.8^{A,c}$ | $22.7\pm1.5^{A,b}$ | $13.7\pm2.0^{A,a}$ | $74.0\pm1.2^{A,e}$ |
| 0.1 | $68.3\pm1.9^{B,d}$ | $53.7\pm2.0^{B,c}$ | $29.0\pm2.1^{A,b}$ | $20.0\pm2.3^{A,a}$ | $85.3\pm0.9^{B,e}$ |
| 0.2 | $82.7\pm1.3^{C,d}$ | $71.3\pm1.5^{C,c}$ | $40.7\pm1.5^{B,b}$ | $28.7\pm1.5^{B,a}$ | $94.0\pm1.2^{C,e}$ |
| 0.3 | $91.3\pm1.8^{D,d}$ | $82.0\pm1.2^{D,c}$ | $57.3\pm2.0^{C,b}$ | $39.0\pm1.7^{C,a}$ | $95.3\pm1.2^{C,d}$ |

Values represent the mean±SE of three repeated experiments with five replicates each. Values followed by either the same capital letters within a column or the same small letters within a row are not significantly different ($P<0.05$) according to Tukey's test.

**Table 2 Antimicrobial activity of anise extracts against selected pathogens**

| Extract | Concentration (mg/ml) | Zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | *Bacillus cereus* | *Staphylococcus aureus* | *Salmonella typhimurium* | *Escherichia coli* |
| Ethanolic extract of seeds | 1.25 | $7.3\pm0.9^{A,a}$ | $6.0\pm0.6^{A,B,C,a}$ | ND | ND |
| | 2.5 | $12.3\pm0.7^{B,b}$ | $9.7\pm1.2^{C,b}$ | $4.3\pm0.9^{A,a}$ | $3.3\pm0.9^{A,a}$ |
| <aname="_Hlk527884965"> | 5 | $21.0\pm1.2^{C,b}$ | $18.3\pm1.5^{D,b}$ | $9.7\pm1.2^{B,a}$ | $7.0\pm1.2^{B,a}$ |
| Aqueous extract of seeds | 1.25 | $3.3\pm0.7^{A,a}$ | $2.7\pm0.3^{A,a}$ | ND | ND |
| | 2.5 | $7.0\pm1.2^{A,a}$ | $7.3\pm0.9^{B,C,a}$ | ND | ND |
| | 5 | $13.7\pm1.5^{B,b}$ | $14.3\pm1.2^{D,b}$ | $4.0\pm0.6^{A,a}$ | $2.7\pm0.7^{A,a}$ |
| Ethanolic extract of aerial parts | 1.25 | ND | ND | ND | ND |
| | 2.5 | ND | ND | ND | ND |
| | 5 | $4.3\pm0.9^{A,a}$ | $4.0\pm0.6^{A,B,a}$ | ND | ND |
| Aqueous extract of aerial parts | 1.25 | ND | ND | ND | ND |
| | 2.5 | ND | ND | ND | ND |
| | 5 | ND | ND | ND | ND |
| Tetracycline | 0.5 | 26.7±0.9 | 26.0±0.6 | 22.0±1.2 | 21.3±1.2 |

Values represent the mean±SE of three repeated experiments with five replicates each. Values followed by either the same capital letters within a column or the same small letters within a row are not significantly different ($P<0.05$) according to Tukey's test. ND, not detected.

The data indicates the resistance of some microorganisms to solvent extracts of aerial parts. While the ethanolic extract of aerial parts displayed inhibitory activity against the growth of only *B. cereus* and *S. aureus*, the aqueous extract of aerial parts failed to show any effectiveness. It was observed that the antibacterial activity of extracts increased with an increase in their concentrations. Statistical analysis demonstrated that Gram-positive bacteria (*S. aureus* and *B. cereus*) were more sensitive to anise extracts than the Gram-negative ones (*S. typhimurium* and *E. coli*). In this research, tetracycline, which was used as positive control, showed strong antimicrobial activities against the tested Gram-positive and Gram-negative bacteria, with inhibition zones ranging from 21.3±1.2 to 26.7±0.9 mm.

## Discussion

In recent years, much attention has been devoted to natural bioactive compounds and their health benefits. Plant extracts, in particular, are commonly rich in phenolic compounds such as flavonoids, phenolic acids, and tannins that have multiple biological effects including antioxidant and antimicrobial properties [20]. Phenolic compounds are hydrogen donors capable of directly scavenging free radicals and reducing oxidative damage [21,22], which makes them potent antioxidants. In addition to the antioxidant activity, phenolic compounds act as antimicrobial agents via several mechanisms including the disruption of microbial membranes [23,24]. In the present study, the antioxidant and antimicrobial activities of anise extracts may be attributed to their phenolic contents since numerous phytochemical studies indicated the presence of noticeable amounts of phenolic compounds in anise [25,26]. Perhaps, this might be also a possible explanation of our second observation that the increase of solvent extract concentration is accompanied by a parallel increase in their antioxidant and antimicrobial activities. Our results are in agreement with those of Hassim *et al.* [27], Pensec *et al.* [28], Mehmood *et al.* [29], and Gabr *et al.* [14] who suggested a positive relationship between the total phenolic content and the biological capabilities (antioxidant and antimicrobial activities) of the plant extracts.

In this investigation, solvent extracts prepared from the seeds of anise exhibited a significantly higher activity than those prepared from the aerial parts and of anise. The varied effects of extracts from different parts of anise could be attributed to the differences in their phytochemical constituents. Different parts contain different bioactive compounds at different levels. This result is in consistent with that of Embong and his colleagues, who investigated the components of the whole plants and the seeds of *Pimpinella anisum* and reported that the major oil constituent (the flavonide *trans*-anethole) – which is widely known to have strong biological activities – was 57.4% of whole plant and 75.2% of seed oil [30]. A similar trend was observed also in other medicinal plants such as chicory. Jurgonski and his colleagues investigated the chemical composition of the seed, peel, leaf, and root extracts and found that the seed extract was the richest source of minerals, fat, protein, and most importantly, phenolic compounds [31].

Solvent types may also affect the biological activities of the extracts. Solvents differ in the extraction capabilities depending on their polarity and on the solute's chemical structure. Different solvent extracts have different soluble phytoconstituents in different amounts and hence, they have varying degrees of biological activities. In the present study, ethanol extracts demonstrated higher activity compared with the corresponding water extracts. This result suggests that most of the bioactive constituents in anise are soluble in ethanol. For example, anethole (the principal active constituent in anise essential oil) is known to be slightly soluble in water whereas it exhibited high solubility in ethanol. Our result is in contrast with the report of Gülçin *et al.* [32] who indicated the superiority of aqueous extracts of anise over ethanolic extracts. It is important to note that the comparison of the findings of diverse studies is complicated by the fact that there are many factors contributing to the differences in the biological activity of the same solvent. The status of plant raw material (culture conditions, harvesting time, storage, etc.), the type of plant material (fresh, frozen, or lyophilized), the mode of extraction (using heat or cold), or the extraction conditions (extraction time, extraction temperature, pH, etc.) may significantly influence the same solvent activity [33–37].Results on the sensitivity of the tested bacteria to anise extracts showed that Gram-positive bacteria were found to be more sensitive than Gram-negative ones. This behavior might be attributed to the differences in their cell wall structures. Gram-negative bacteria have an extra hydrophilic outer membrane that functions as a preventive barrier against hydrophobic compounds and inhibits the accumulation of phenolic compounds in the target cell membrane [38]. This renders the Gram-negative bacteria generally more resistant to plant extracts than the Gram-positive bacteria [39]. Our results are in accordance with many studies that have reported on the antimicrobial potentiality of anise against Gram-positive bacteria, Gram-negative bacteria, yeast, and filamentous fungi [40–42].

## Conclusion

Anise extracts show a satisfactory antioxidant and antimicrobial powers suggesting their potential use to treat infectious diseases and to fight free radicals. The results demonstrate the superiority of seed extracts over aerial part extracts. The results also indicate the stronger activity of ethanolic extracts compared with aqueous extracts. This research provides scientific insights into the antioxidant and the antimicrobial potency of anise. Further phytochemical and pharmacological studies are needed to determine the specific component(s) responsible for the biological activity and address the safety and toxicity issues.

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**Conflicts of interest**

There are no conflicts of interest.

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