



Full Length Article

Characteristics of *Anethum graveolens* (Umbelliferae) Seed Oil: Extraction, Composition and Antimicrobial Activity

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ABSTRACT

Plant essential oils are potential source of antimicrobials of natural origin. The essential oils were extracted from the *Anethum graveolens* seeds by steam distillation by placing the seeds in retort and passing pressurized steam through it. The temperature of the distillation flask was kept above 100°C with the help of a sand bath to avoid the condensation of vapors inside the flask. The steam and oil vapors mixture was then condensed and collected in a receiver kept in ice water in order to prevent the evaporation of low boiling constituents of the oil. The essential oil was then extracted from the condensed distillate in the separator. The oil smelled like grass and was pale yellow in color, with a watery viscosity. Percentage yield, specific gravity, refractive index and acid value of oils were 0.66%, 1.51, 1.49 and 0.58, respectively. The oils showed antimicrobial activity (100%) as compared with oxytetracycline (100%), chloramphenicol (35%), gentamicin (81.82%) and penicillin G (57.14%) against *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis* and *Staphylococcus aureus*, respectively. Capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid and arachidic acid as heterogeneous components from *Anethum graveolens* oils were separated using gas chromatography.

Key Words: *Anethum graveolens*; Sowa; Dill seed; Essential oil; Antimicrobial activity; Gas chromatography

INTRODUCTION

History of essential oils are very ancient, the Egyptians, Hindus, Greeks and Arabs were much familiar with extraction and use of essential oils. In those times distillation was the only method used for their extraction, actual yield of oil extraction and characterization started in early 1920s. Plant essential oils are potential source of antimicrobials of natural origin (Valero & Giner, 2006).

Essential oil plants include a wide range of plant species, mainly used in the preparation of perfumes, cosmetics, beverages, medicinal foods, disinfectants, insecticides, fungicides, smoking, chewing, tobacco and condiments. Essential oils are extracted from aromatic plants of many genera, which are distributed worldwide. These oils are found in various parts (seeds, leaves, fruits barks & roots) of aromatic plants. English people name *Anethum graveolens* as dill and in subcontinent it is called as sowa. It is used as flavoring and preservative agent. Its medicinal uses are as an antispasmodic, carminative, diuretic, stimulant and stomachic (Simon *et al.*, 1984). Some of the earlier studies had shown the antimicrobial activity of *Anethum graveolens* against *Saccharomyces cerevisia* and *Listeria monocytogenes* (Pascal *et al.*, 2002). Keeping in view this fact it was hypothesized that *Anethum graveolens* can have antimicrobial activity against other microbes.

Various workers had used different methods of extracting oils like solvent extraction and steam distillation. Solvent extraction gives very low yield of essential oil and non-volatile components like waxes and pigments, while steam distillation is used in the manufacture and extraction of essential oils. In the present study, essential oils of *Anethum graveolens* were extracted first time in Pakistan by modified steam distillation method and its antimicrobial activity was tested against *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis* and *Staphylococcus aureus*. Moreover, heterogeneous components from this oil were separated by gas chromatography.

MATERIALS AND METHODS

Plant material. Mature seed of *Anethum graveolens* were purchased from the local market. The seeds were sieved, washed with water and dried in open.

Recovery and physico-chemical properties of essential oil. The essential oil was recovered from dry seeds by modified steam distillation (Masango, 2005) and physico-chemical properties like percentage yield of essential oil (Fiestone, 2000), specific gravity at room temperature (32°C) using specific gravity bottle (Prosky, 2000), index of refraction by Abbe's refractometer, taking water as a reference (Woodbury, 2000) and acid value were determined. For acid value one gram of essential oil was

mixed with 50 mL of neutral alcohol and heated in water bath for 10 min. The contents of the flask were titrated against 0.1 N potassium hydroxide (KOH) solution using phenolphthalein as an indicator (Bradley, 2000).

Acid value = $56.1 \times \text{Normality of KOH solution} \times \text{Volume of KOH used (mL)}$.

Determination of antimicrobial activity. Antimicrobial activity was determined by the size of inhibition zones on agar plates (Aggarwal *et al.*, 2001) using local isolates of *E. coli*, *S. typhi*, *B. subtilis* and *Staph. aureus*. These organisms were obtained from Department of Veterinary Microbiology, University of Agriculture, Faisalabad, Pakistan.

The standard antibiotic discs (containing 30 ug of the antibiotic on each disc) of penicillin G (for *Staph. aureus*), oxytetracycline (for *E. coli*), chloramphenicol (for *S. typhi*) and gentamicin (for *B. subtilis*) were prepared and used as control. The discs of original dill seed essential oil and its dilutions (1:10, 1:50, 1:100 & 1:200) were made in distilled water using 10% surfactants (Span-80 as oil phase & Tween-80 as water phase) in the test oil to reduce surface activity and stabilize emulsion (Southwell *et al.*, 1993). The discs were dried in hot air oven at 70°C (Bauer *et al.*, 1966).

Nutrient agar was prepared, autoclaved and dispensed in sterilized petri plates. Simultaneously, nutrient broth was prepared in test tubes and autoclaved. The sterility of media was checked at 37°C for 24 h in an incubator (Cruickshank *et al.*, 1975). Sterilized nutrient broth was inoculated with test bacteria and incubated at 37°C for 24 h. The organisms were confirmed morphologically using Gram's staining. Then from each tube 0.1 mL of broth culture of organisms was uniformly inoculated on nutrient agar plates. Discs of test oil and its dilutions along with standard antibiotics were placed on nutrient agar plates previously seeded with test bacteria. Zones of inhibition due to activity of test oil, its dilutions and antibiotics were measured after 24 h of incubation at 37°C. The diameters of zones of inhibition (in mm) were measured (Aggarwal *et al.*, 2001).

Chromatographic analysis. Gas chromatography was performed for separation of heterogeneous components from dill seed oil. Chromatogram produced was compared with the chromatogram of the standard compounds (C₁₀-C₂₀) of dill seed (Lane, 2000).

RESULTS AND DISCUSSION

The essential oils from the *Anethum graveolens* seed by modified steam distillation possessed yellowish color and pleasant odour. The maximum percentage yield (0.66%) was obtained after 13 h of distillation. Specific gravity of essential oil was 1.51 and it showed a good index of purity of oil. The refractive index at 32.5°C was 1.49 and this property was helpful in the identification of oils and was also used to determine purity. The refractive index increases with un-saturation and decreases with the high percentage of free fatty acids. The oil showed low amount of acids (0.58)

Table I. Zones of inhibition in mm against standard microorganisms

Test discs	<i>E. coli</i>	<i>B. subtilis</i>	<i>Staph. aureus</i>	<i>S. typhi</i>
Antibiotics	8 ^a	11 ^b	14 ^c	20 ^d
Original Oil	8	9	8	7
1:10*	7	8	8	7
1:50 *	6	7	6	8
1:100*	4	6	5	4
1:200*	--	1	--	--

^a Oxytetracycline, ^b Gentamicin, ^c Penicillin G, ^d Chloramphenicol, *Dilution

Table II. Fatty acids and their concentration in *Anethum graveolens* seed oil extracted through steam distillation method

Peak No.	Retention time	Name	No. of carbons	Concentration (%)
1	0.882	Capric acid	C ₁₀	5.97
2	1.508	Lauric acid	C ₁₂	1.29
3	1.96	Myristic acid	C ₁₄	0.25
4	3.043	Palmitic acid	C ₁₆	4.66
5	3.981	Stearic acid	C _{18:0}	3.86
6	5.913	Oleic acid	C _{18:1}	37.05
7	7.798	Linoleic acid	C _{18:2}	45.13
8	13.636	Linolenic acid	C _{18:3}	0.26
9	17.96	Arachidic acid	C ₂₀	1.32

giving information only about its condition, while gas chromatographic analysis revealed its composition.

Antimicrobial activity of dill seed oil and its various dilutions were compared with the standard antibiotics. Oxytetracycline was used as standard antibiotic against *E. coli* which showed 8 mm zone of inhibition, which was same by the original oil. The 1:10, 1:50, 1:100 dilution of oil gave 7, 6 and 4 mm zone of inhibition, respectively. The antimicrobial activity of dilution 1:200 was negative against *E. coli* (Table I).

Gentamicin was used as standard antibiotic against *B. subtilis*, which showed 11 mm zone of inhibition, while the original oil showed 9 mm zone of inhibition. The 1:10, 1:50, 1:100 and 1:200 dilutions of oil showed 8, 7, 6 and 1 mm zone of inhibition, respectively (Table I).

Penicillin G was used as standard antibiotic against *Staph. aureus*, which showed 14 mm zone of inhibition, while original oil and its dilutions 1:10, 1:50, 1:100 showed 8, 8, 6 and 5 mm zone of inhibition, respectively. The antimicrobial activity of dilution 1:200 was found as negative against *Staph. aureus* (Table I).

Chloramphenicol was used as standard antibiotic against *S. typhi* which showed 20 mm zone of inhibition, while original oil and its dilutions 1:10, 1:50, 1:100 showed 7, 7, 8 and 4 mm zone of inhibition, respectively. The antimicrobial activity of dilution 1:200 was also found negative against *S. typhi* (Table I).

Three different methods agar disc diffusion method without stabilizing agent (Alvaro *et al.*, 2003; Alma *et al.*, 2004; Salehi *et al.*, 2006) or with stabilizing agent (Carson & Riley, 1995), minimum inhibitory concentration by micro titration plate (Alma *et al.*, 2004) and kill time studies

(Alvaro *et al.*, 2003) have been tried to test antimicrobial activity of the essential oils against various microorganisms.

Various essential oils obtained from the plants showed antimicrobial activity against a range of microorganisms including Gram's positive bacteria, Gram's negative bacteria and fungi. However, the differences may be explained by susceptibility, testing conditions, physico-chemical characteristics of the oil and strain differences. The antimicrobial activity of volatile oils has been observed by Singh *et al.* (2002). The earlier studies on essential oil of *Anethum graveolens* had some antimicrobial activity against *Saccharomyces cerevisia* and *Listeria monocytogenes* (Pascal *et al.*, 2002). However, in present study *E. coli*, *S. typhi*, *B. subtilis* and *Staph. aureus* were used. The antimicrobial activity of *Anethum graveolens* has also been reported by Aggarwal *et al.* (2001) and the result of this study further confirms the antimicrobial activity of *Anethum graveolens*.

Jirovetz *et al.* (2003) performed gas chromatography and gas chromatography mass spectrophotometry of dill seed essential oil and they also observed the antimicrobial activity of the oil. Delaquis *et al.* (2002) separated heterogeneous mixture of compounds from essential oil of dill by fractional distillation and analyzed by gas chromatography mass spectrophotometry. The chemical analysis of dill seed oil was carried out by gas chromatography, which revealed the presence of various fatty acids namely capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid and arachidic acid. The various fatty acids present in *Anethum graveolens* oil and their percentage concentrations are shown in Table II.

Fatty acids are saturated and unsaturated depending upon the double bond. *Anethum graveolens* oil contained saturated fatty acids namely: capric (decanoic), lauric (dodecanoic), myristic (tetradecanoic), palmitic (hexadecanoic) and stearic acids (octadecanoic), while unsaturated fatty acids were oleic, linoleic, linolenic and arachidic acids. The differences in geometry between various types of unsaturated fatty acids, as well as between saturated and unsaturated fatty acids, play an important role in biological processes and in the construction of biological structures such as cell membranes. Fatty acids act as anionic detergents and literature on this effect is as far back as reported by Clark (1899). Much of the early literature on this aspect can be found in reports by Nieman (1954). In subsequent years the antifungal and bactericidal properties of fatty acids have been extensively investigated (Prince, 1959). Other reports point to the inactivation of virus by various soaps (Kabara *et al.*, 1972). Lauric acid had the most bacteriostatic activity on Gram positive organisms (Prince, 1959). The addition of double bond increased this activity of fatty acids (Fuller & Moore, 1967) and the addition of second double bond further increases the toxicity of compound to Gram positive bacteria. The toxic activity of fatty acids increases in order: oleic < linoleic < linolenic

acid. It is important that free carboxylic group is necessary for bactericidal activity because ester formation generally decreases this activity of fatty acids (Wyss *et al.*, 1945).

Reduction of the carboxylic group to aldehyde or alcohol or change to an amine or amide group increases bacteriostatic effects. Capric acid is used in manufacture of perfumes, lubricants, greases, rubber, dyes, plastics, food additives and pharmaceuticals. Lauric acid is used in soaps and shampoos and also to raise metabolism. It is believed that 20% thyroidal hormones activation occurs from lauric acid. Myristic acid is used in cosmetic and topical medicinal preparations where good absorption through the skin is desired. Palmitic acid is an antioxidant. Stearic acid is an ingredient in making candles, soaps, plastics, oil pastels, cosmetics and for softening rubber. Oleic acid makes up 55-80% of olive oil. Linoleic acid is also used in making soaps, emulsifiers and quick-drying oils. Arachidic acid is present in phospholipids of membranes of body cells especially in the brain and is involved in cellular signaling. Arachidic acid is required dietary part of those mammals which lack the ability to convert linoleic acid into arachidic acid. Alpha-linolenic acid is an essential dietary requirement of all mammals and 2 to 3 g per day prevent primary and secondary heart diseases and eczema, while gamma-linolenic acid has anti-inflammatory properties.

CONCLUSION

Anethum graveolens essential oil has antimicrobial anti-microbial activity against *E. coli*, *S. typhi*, *B. subtilis* and *Staph. Aureus*, which can be profitably exploited.

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