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Full Length Research Article

Phytochemical Screening and GC-MS Analysis of Bioactive Constituents of Methanolic Extract from *Anethum graveolens* Leaves Powder

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Abstract

Anethum graveolens leaves are the medicinal plants belong to the umbelliferae family and native to Mediterranean, Europe and Asian countries. The dill leaves are smaller and similar to the fennel leaves. The phytochemicals are the compounds that possess many pharmacological activities. Dill is a plant that is known for their medicinal values. This plant preliminary phytochemical screening was found to have positive reactions on carbohydrates, alkaloids, terpenoids, flavonoids, tannins, cardiac glycoside. In this study the possible phytochemicals were investigated of dried dill leaves powder with methanolic solvent extraction of the sample using GC-MS method. Results revealed that the phytochemicals confirmed the presence of biological activity including antimicrobial, anti-cancer, diuretic, anti-inflammatory, hypocholesterolemic, antiarthritic, and antioxidant.

***Key words*:** Phytochemicals, *Anethum graveolens*, GC-MS, Accelerated solvent extractor, Air dried

Anethum graveolens L. is the sole species of the genus Anethum, though classified by some botanists in the related genus Peucedanum as *Peucedanum graveolens* (L.) [7]. *Anethum* grows up to 90 cm tall, with slender stems and alternate leaves finally divided three or four times into pinnate sections slightly broader than similar leaves of fennel. The yellow flower develops into umbels [12]. Some pharmacological effects of *Anethum graveolens* have been reported such as antimicrobial [4] antihyperlipidemic and antihypercholesterolemic activities [13]. Dill is a crucial aromatic herb used to savor various foods, including salads, sauces, soups, and seafood [11]. Plant extracts together with their phytochemicals possess antimicrobial properties which are of great importance for therapeutic treatment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. There has been incredible improvement in medicinal plant research which involve the isolation and identification of secondary metabolites of plants and their use as active principles in therapeutics. Herbs and plants and their parts have been used for medical or therapeutic purpose long before and the use of plants and their parts in the treatment of diseases has gained considerable importance in recent past. The purpose of this study was to determine the chemical constituents of dried dill herb powder. The literature shows that there are no previous reviews on the phytochemical composition of methanolic extract of dill herb powder using GC-MS.

MATERIALS AND METHODS

Extraction of plant material

The fresh dill leaves were collected from Ernakulam district of Kerala. The plants were identified by Dr. A. K. Pradeep, Assistant Professor, Department of Botany, Calicut University. The specimen No. 148276 of the plant was deposited in the University Herbarium. The fresh leaves were separated from the stem. The fresh leaves were washed under running tap-water and drained for 5 minutes. Then the drained leaves were subjected to shade drying on room temperature on a dry muslin cloth. The leaves were dried about to 7 days under normal air passage. The dried leaves were grounded in a mixer grinder to get fine powder. About 9 gram of powdered dill leaves were added to 200ml of methanol and kept under accelerated solvent extraction. The solvent extraction was performed at Central Instruments Laboratory, College of Veterinary and Animal Science, Mannuthy, Kerala. The obtained methanol extract was used for GC-MS analysis.

Phytochemical screening

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The phytochemical screening of the *Anethum graveolens* leaves was determined by using standard methods [9] to identify the chemical composition was done at the chemistry lab of MES Keveeyam College, Valanchery. The phytochemical present in *Anethum graveolens* leaves were illustrated in the (Table 1).

Table 1 Procedure and inference of phytochemical screening

Phytochemical	Procedure	Observation	Inference
Alkaloids	Mayer's test Few ml of plant extract+ 2 drops of Mayer's reagent	White creamy precipitate Yellow coloured precipitate	Presence
Terpenoids	5ml extract+ 2ml chloroform+ 3ml conc. H_2SO_4	Reddish brown colour at interphase	Presence
Flavonoids	Extract few ml+ conc. H_2SO_4	Yellowish orange colour	Presence
Cardiac glycoside	5ml extract+ 2ml of glacial acetic acid+ 2-3 drops ferricchloride +1ml conc.H_2SO_4	Appearance of green ring first turns violet then to brown	Presence
Tannin	Extracts+ 10% ferric chloride	Green or blue colour	Presence

GC-MS analysis

GC-MS has been used for the analysis of biological samples for several decades. This technique is used by the US National Institute of Standards and Technology and other agencies for the development of definitive methods to qualify standard reference materials and assign accurate concentration to reference materials of many clinically relevant analyses, including cholesterol, glucose, steroid hormones, creatinine, and urea nitrogen. One of the most common applications of GC-MS is drug testing for clinical or forensic purposes. Many drugs have relatively low molecular weight and nonpolar and/or volatile properties, making these compounds particularly suitable for analysis by GC [1].

GC-MS analysis was performed at Central Instruments Laboratory, College of Veterinary and Animal Science, Mannuthy, Kerala. For GC-MS analysis of plant extract, Triple Quadruple GCMSMS TSQ8000MSMS system was utilized. Column: DB-5MS; GC oven temperature was programmed to 110℃ and hold for 2 minutes. Then the temperature was raised to 150℃ at a rate of 15℃/min and hold at 115℃for 1 minute. Again, the temperature was raised to 250℃ at a rate of 10℃/min and hold at 250℃ for 5 minutes. Totally it took 20.67 minutes. The GC-MS data is shown in (Table 2).

RESULTS AND DISCUSSION

Phytochemicals have been reported to have many nutritive, biological and therapeutic properties [2]. They serve as useful taxonomic markers in identifying particular species as well as to distinguish it from related species, hence are helpful in the delimitation of taxa [6]. The results pertaining to GC-MS analysis of the methanolic extract of *Anethum graveolens* lead to identification of a number of compounds. These compounds were identified through mass spectrometry attached with GC (Fig 1).

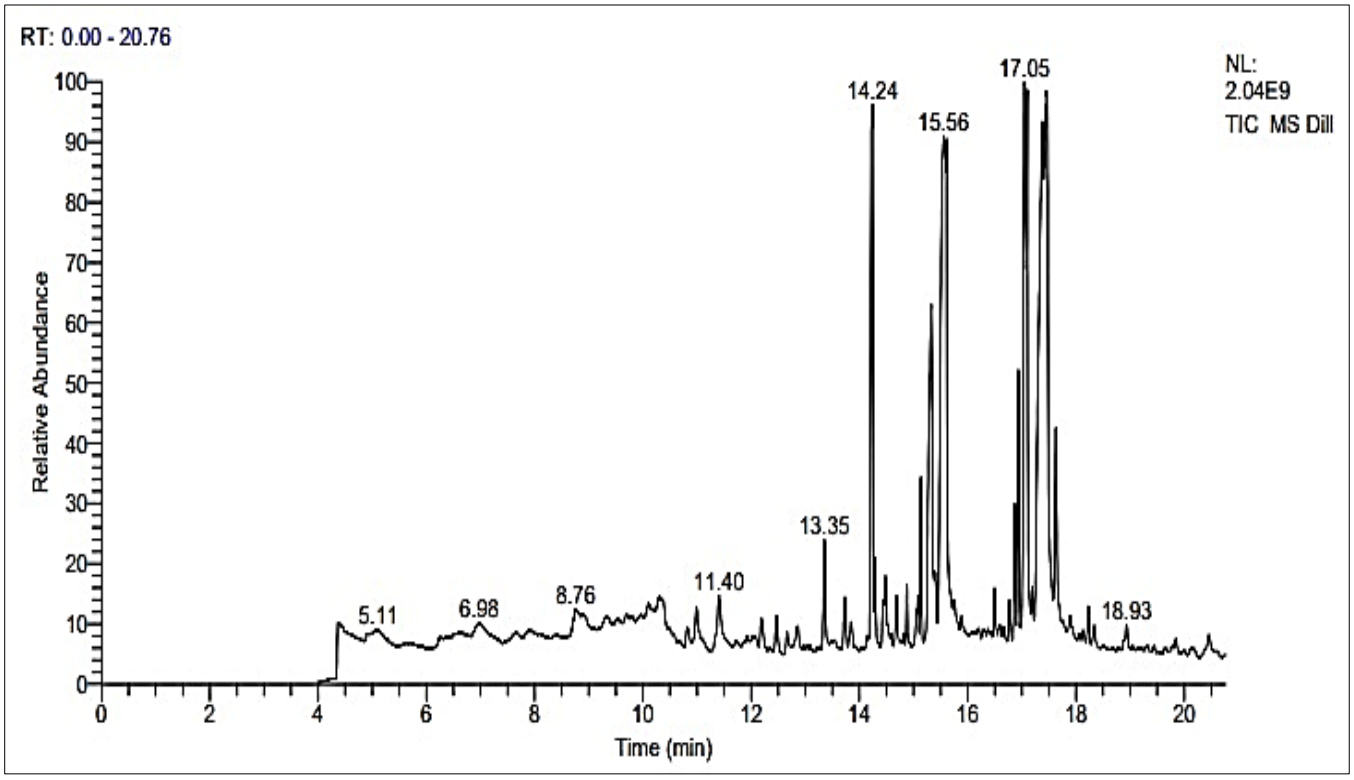
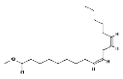
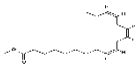
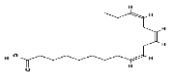


Fig 1 GC-MS chromatogram of methanolic extract of *anethum graveolens* leaves powder

The active principles with their retention time (RT), molecular formula, molecular weight (MW), concentration (%) and structure are presented in (Table 2). Sixteen compounds were identified in the GC-MS analysis. The major components presented in the *Anethum graveolens* (dill leaves) are listed in the order of their peak. Their compound name, retention time, molecular formula, molecular weight, peak area and structure are presented in (Table 2).

Table 2 Phytochemicals identified in the methanol extract of the *Anethum graveolens* leaves by GC-MS

S. No	Retention time	Name of the compound	Molecular formula	Molecular weight	Peak area (%)	Structure
1	4.36	Erythritol	$C_4H_{10}O_4$	122	1.54	
2	11.40	3-Methyl-4-phenyl-1H-pyrrole	$C_{11}H_{11}N$	157	1.22	
3	13.35	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	1.41	
4	14.24	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	8.71	
5	14.24	Phytol, acetate	$C_{22}H_{42}O_2$	338	8.71	
6	15.14	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.50	
7	15.32	9,12,15-Octadecatrienal	$C_{18}H_{30}O$	262	7.56	
8	15.56	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	13.88	
9	15.61	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	2.28	
10	16.87	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	1.31	
11	16.94	9,12,15-Octadecatrienoic acid, methyl ester, (z,z,z)-	$C_{19}H_{32}O_2$	292	2.58	
12	17.05	Phytol	$C_{20}H_{40}O$	296	9.42	
13	17.10	1-Hexadecen-3-ol, 3,5,11,15-tetramethyl-	$C_{20}H_{40}O$	296	3.01	
14	17.38	9-Octadecynoic acid	$C_{18}H_{32}O_2$	280	13.08	
15	17.45	9,12,15-Octadecatrienoic acid, (z,z,z)-	$C_{18}H_{30}O_2$	278	9.70	
16	17.63	Octadecanoic acid	$C_{18}H_{36}O_2$	284	2.15	

Based on the interpretation of the analysis the leaves powder consists of the components n-hexadecanoic acid (13.88), 9-Octadecynoic acid (13.08), 9,12,15-Octadecatrienoic acid, (z,z,z)- (9.70), Phytol (9.42), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (8.71), Phytol acetate (8.71), 9,12,15-Octadecatrienal (7.56), 1-Hexadecen-3-ol, 3,5,11,15-tetramethyl- (3.01), 9,12,15-Octadecatrienoic acid, methyl ester, (z,z,z)- (2.58), Pentadecanoic acid (2.28), Octadecanoic acid (2.15), Erythritol (1.54), Hexadecanoic acid, methyl ester (1.50), Tetradecanoic acid (1.41), 9,12-Octadecadienoic acid, methyl ester (1.31), 3-Methyl-4-phenyl-1H-pyrrole (1.22) and various other compounds are found in very low levels. These phytochemicals are responsible for various pharmacological actions like osmoprotectant, antioxidant, antimicrobial, anti-inflammatory, anticancer, diuretic, hypocholesterolemic, 5-Alpha reductase inhibitor activity, antitumor, chemopreventive, neuroprotective and hepatoprotective (Table 3) [15-16]. *Anethum graveolens* leaves have medicinal value with the presence of these major constituents.

Table 3 Biological activity of phytochemical constituents present in methanolic extract of dill leaves powder

S. No.	Name of the compound	Compound nature	**Activity
1	Erythritol	Sugar alcohol	Osmoprotectant Antioxidant Insecticidal (10) Improve blood vessel function in people with type 2 diabetes reduces oxidative stress and reverses endothelial dysfunction [3], [5]
2	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Terpene alcohol	Antimicrobial
3	Phytol, acetate	Diterpene compound	Antimicrobial Anti-inflammatory Anticancer Diuretic
4	Hexadecanoic acid, methyl ester	Fatty acid ester	Anti-inflammatory, antioxidant, inhibit production of uric acid activity, urine acidifier, mainly used to produce soaps, cosmetics, release agents, antifibrinolytic, heamolytic, lubricant, nematicide, antialopecic and antidepressant [8]
5	n-hexadecanoic acid	Saturated fatty acid	Antioxidant Hypocholesterolemic Anti-inflammatory 5-Alpha reductase inhibitor activity
6	9,12-Octadecadienoic acid, methyl ester	Linoleic acid ester	Hypocholesterolemic Nematicide Antiarthritic Hepatoprotective Antiandrogenic 5-Alpha reductase inhibitor Antihistaminic Insectifuge Antieczemic Antiacne [14]
7	Phytol	Diterpene	Anticancer, Antioxidant, Anti-inflammatory, Diuretic, Antitumor, Chemopreventive, Antimicrobial, use in vaccine formulations
8	9,12,15-Octadecatrienoic acid, (z,z,z)-	Fatty acid ester	Antimicrobial Reduce complications in covid-19 patients Neuroprotective Anti-inflammatory Anticancer Hepatoprotective Antioxidant Hypocholesterolemic
9	Octadecanoic acid	Fatty acid ester	Play role in food reward Lowers HDL cholesterol

Anethum graveolens,

*Activity source: Dr. Duke's phytochemical and ethnobotanical databases

CONCLUSION

The present work has been performed to analyze the various phytochemicals present in the methanolic extract of *Anethum graveolens* leaves powder and to determine their pharmacological properties which could serve as an important finding for the upcoming researches on the phytochemical properties in newly developed drugs. From the above discussion it is obvious that the compounds of methanolic extract of *Anethum graveolens* leaves powder have diverse effect on medicinal and industrial applications. Therefore, this primary information will facilitate in conducting further studies to utilize their pharmacological properties in vivo studies and demonstration of their safety and efficacy in clinical trials.

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Conflict of interests

The author hereby declares no conflict of interest regarding the manuscript and documentation done.

Abbreviation used

GC MS: Gas chromatography- mass spectrometry.

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