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GC-MS Analysis and Biological Effects of Essential Oil from *Trachyspermum ammi L*

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Summary: *Trachyspermum ammi L*, local seed's essential oil and its crude methanolic extract were evaluated for antioxidant, antibacterial, and urease inhibition activities. Largely, 18 constituents were identified in the essential oil using The GC-MS. The promising constituent reported was terpeniodes; which seem to be responsible for the reported biological activities. DPPH Free radica1 scavenging assay was done to evaluate the antioxidant activities of both oil and crude extract. The DPPH scavenging activity results in lC_{50} values of 75 ± 0.05 and 300 ± 0.03. Antibacterial activity of the oil was carried out by using strains of *Escherichia coli* (89%), *Bacillus subtilis* (85%), and *Streptococcus aureus* (80%), while for the crude extract strains of *E. coli* (86%) *B. subtle* (89%), and *S. aureus*; (85%) were used. E. coil of *T. ammi* exhibits promising results with *Aspergillus. flavus*[1] (86%) and *Aspergillus*$_1$ *Niger*[1] (87%). The phytochemical analysis of the *T. ammi*, crude extract showed the presence of saponins, flavonoids, steroids, terpenoids, and cardiac glycosides in the phytochemical investigation. Urease inhibition showed potent inhibition against *B.P* and *J.B Urease* with IC_{50} values of 85 ± 0.06 and 67.45 ± 0.8, respectively. Plant crude showed optimal results for B.P and Jack bean urease with IC_{50} values of 55.77 ± 0.01 and 35.56 ± 0.04, respectively.

Keywords: *T. ammi*, Essential Oil, GC-MS, DPPH scavenging activity, Urease, Antifungal.

Introduction

Trachyspermum ammi L belongs to the family Apiaceae, an indigenous Egypt plant and also cultivated in Pakistan. In Pakistan, the plant's common name is Ajwain, and its seeds are used in folk medicine for treating amenorrhea, stomach disorders, gastrointestinal problems, pharyngitis, and bronchitis. The bioactive constituents from roots of *T. ammi*, have been utilized as a respiratory stimulant for kidneys and heart diseases [1, 2]. Essential oil is one of the traditional sources of fragrance and flavours. In addition, they have bioactive molecules with significant therapeutic influence [3]. The oil presents a rich source of thymol (50%), a strong anti-spasmodic, disinfectant, and fungicide. Thymol is also used in toothpaste and perfumery [4]. Essential oils are liquid lipophilic combos that incorporate the materials liable for the aroma of plants and are produced as secondary metabolites [5]. It is believed that the role of essential oils in plants is to allure pollinators and avoid pathogens then they have antibacterial, antifungal, antiviral, and insecticidal effects. The plant species may also have different chemotypes, which diverges mechanism of action and characterized by the significant constituents of new essential oils [6, 7]. The present investigation will further add knowledge in the relevant field and increase the research spectrum. In the current studies, we analyze the essential Oil through GCMS and investigate the antioxidant, antimicrobial, and enzyme inhibition studies.

Experimental

Chemicals

Methanol, Hexane, DMSO, Sabouraud's dextrose agar, urea, thiourea, J. B. and B. P. urease were purchased from Sigma-Aldrich. All the chemical substances used have been of analytical grade.

Collection of plant and Identification

T. ammi plant was collected in September from the village of Lakki Marwat, Bannu, K.P., Pakistan. Taxonomic identification and confirmation were made in the Department of Botany, University of Science and Technology Bannu. The entire laboratory work was carried out in the Department of Chemistry, University of Science and Technology Bannu. A voucher specimen (BG-300) has been submitted to the herbarium of the University of Science and Technology, Bannu, K.P., Pakistan.

Extraction of plant seeds

The shade-dried seeds have been chopped and ground to powder. The plant material (1 kg) became

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soaked in methanol with occasional shaking at room temperature for 20 days, followed by filtration of crude extract. The filtrate became concentrated under a vacuum using a rotary evaporator to obtain a blackish gummy solid (250 g).

Isolation of Essential Oils

Essential oil from seeds of *T. ammi* was obtained through hydro distillations. In this process, 1000 g of seed material was heated with 2 L distilled water in a 5 L flask, fixed in the Clevenger apparatus. In the evaporation-condensation process, volatile constituents of seeds also evaporated with water vapors. Oils being immiscible with water float on the surface of the water. This process was allowed to run for 4 to 6 hours and repeated in replicates. The oil sample was collected from the condenser snout as a pale yellow liquid, dried using anhydrous sodium sulfate, and kept at 4 ºC for further analysis [8].

GC-MS Analysis

GC-MS analysis of samples was carried out using gas chromatography "Agilent USB-393752" (Agilent Technologies, Palo Alto, CA, USA) with an HHP-5MS 5 % phenyl-methyl-siloxane capillary column (30 m × 0.25 mm × 0.25 µm film thickness; Restek, Bellefonte, PA) outfitted with an Agilent HP-5973 mass selective detector in the electron impact mode (Ionization energy: 70 eV). The dynamic temperature was maintained in the oven retained for 1 minute at 70 ºC, continued by increasing the speed of 6 ºC/min to 180 ºC at 5 minutes, and finally for 20 minutes from 5 ºC to 280 ºC (In a range of 220-290 ºC injector/detector). The flow of 'He" was kept at 1mL/min while the splitless mood was held for the injection mood of samples (1/1000 in pentane, v/v).

Components Identification

By using extensive comparative investigation with the spectral data taken from NARC libraries and different compounds were identified [9]

DPPH Free Radical Scavenging Assay

DPPH free radical scavenging assay was performed according to the procedure [9]. Briefly, in a reaction mixture, 900 µl of DPPH solution was added and mixed with 100 µL of test samples, leading to the concentration of 100, 250, 500, and 1000 µg. The reaction mixtures were mixed well and kept in the dark for 40 minutes at a controlled temperature (37 °C). Spectrophotometric absorbance of the reaction mixture was measured at 517 nm. 3% methanol was used as a blank, while a mixture of 100 µl 3% methanol sample and 900 µL of DPPH were taken as a negative control. Ascorbic acid was used as a positive control.

Antibacterial Assay

The Agar disc diffusion method was performed for the antibacterial activity. Gram-positive and gram-negative bacterial strains, e.g., "*S. aureus* (ATCC 29213), *E. coli* (ATCC 15224), *and B. subtilis* (ATCC 6663), were used [10]. Stock solutions were prepared in DMSO in 2mg/mL concentrations, and in the concerned wells, 100 and 200 µL of each diluted solution was added (control contained= 100 and 200 µL DMSO). Erythromycin was used as a reference drug. Plated were incubated at 37 °C for 24 hrs. % Zone of inhibition was determined, and the diameters in mm were calculated.

Antifungal Assay

The antifungal activity of Essential Oil was performed by the agar dilution method. This essay used "A. niger and A. flavus (ATTC 32611) strains. Briefly, the sample was dissolved in DMSO, 25mg/ml, and incubated for seven days at 37 °C. I placed a water pan in an incubator to control humidity. Fungal linear growth was measured in mm concerning negative control and determined the media growth [11].

Urease Inhibition Assay

A urease inhibitory test was carried out to determine ammonia production by indophenol's method. 100 µL of (B.P Urease*) Bacillus pasteurii* urease and (J.B Urease) Jack bean urease were mixed in 100 mM urea 300 µL buffer and then for 15 min incubated at 30°C. Briefly, 300 micro/L of alkali reagent (0.5 %w/v NaOH & 0.1parsent active chloride NaOCl) and 200 micro/L of Phenol Reagent (1% w/v phenol and 0.005 %w/v sodium nitroprusside) mixed to each well. The absorbance was measured at 630 nm after 50 mins. The reaction was performed in triplicate in a final volume of 1000 µL [8].

Results and Discussion

GC-MS analysis of Essential Oil *from seeds of T. ammi*

By Gas Chromatography (G.C.), 18 peaks were recorded in which all compounds were analyzed and quantified in Table-1 (Fig-1). By comparison with the accessible literature, data from the GC-MS system of NARC and the NARC online library, various determinations were achieved. The most prominent polyphenolic components were Pregn-5-en-3-ol, which was 100%, while 21-Bromo-20-methylpregn-5-en-3-*β*-ol was detected at 95% in abundance. The other components obtained were 6H-Benzo[g]-1,3-

benzodioxolo-[5,6-a]-quinolizine-5,8,13,13a-tetrahydro-9,10-dimethoxypregn-5-en-3-ol (87%), Cyclopentadecane (77%), 1,2-Benzene dicarboxylic acid, mono(2-Ethylhexyl) ester (79%), (Z,Z)-(9,12-Octadecadienoic acid (71%), (Z)-9,17-Octadecadienal (72%), Octadecanoic acid (72%),n-Hexadecanoic acid (67%), 4-hydroxy-3,5-dimethoxybenzoic acid hydrazide (61%), n-Decanoic acid (27%), E-4-(-3-Hydroxy-1-propenyl)-2-methoxy phenol (61%), 4-methyl-5-propylnonane (61%), Heptanoic acid (63%), 8-Pentadecanol (59%), 6-bromo-(61%) and 6H-Purin-6-one, 2-amino-1,7-dihydro hexanoic acid (89%). The quality and quantity of reported essential oils components varies with alteration in harvesting periods, drying conditions, chemo, and genotype of the species.

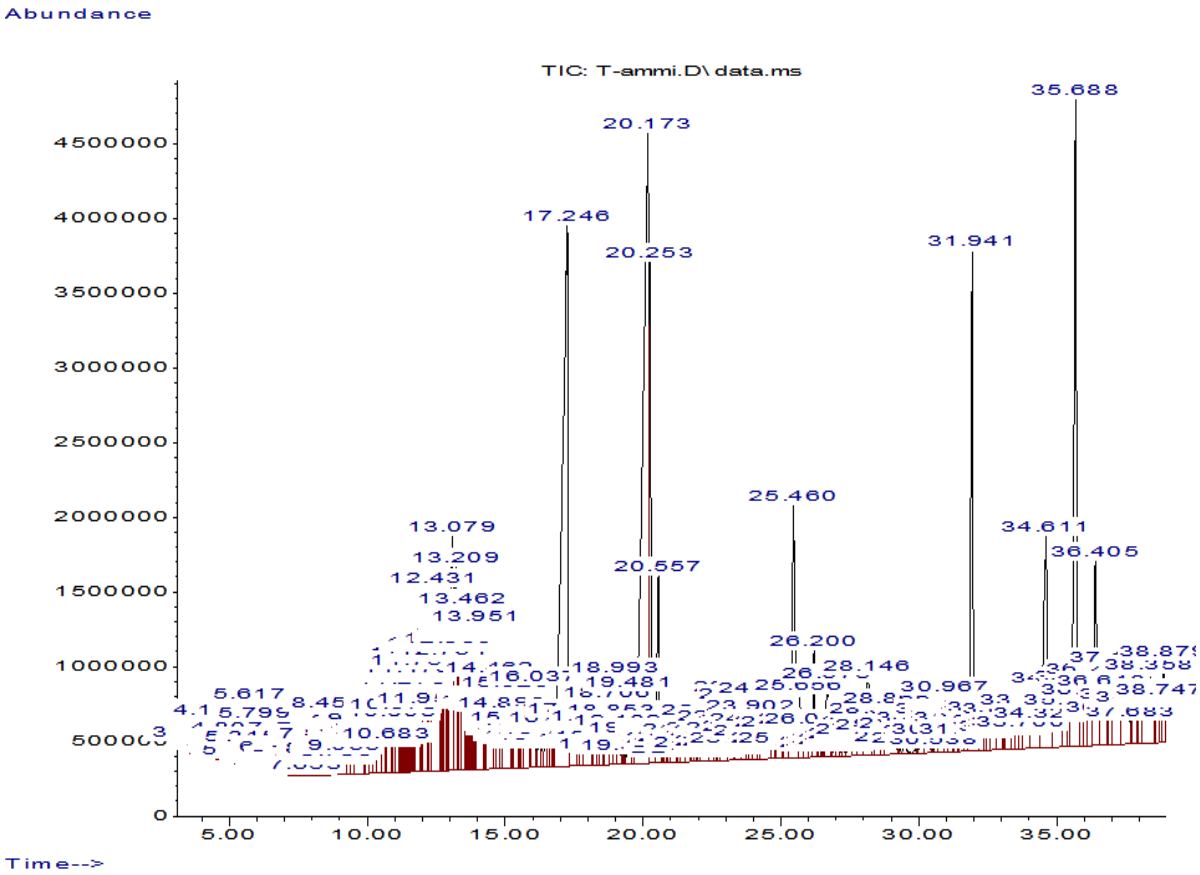

Fig. 1: Total Ion chromatogram for *T. ammi* Essential Oil.

Table-1: GC-MS analyses of *T,ammi* Essential Oil.

S.No	R.I	Compound name	Molecular Formula	% abundance
1	35.68	Pregn-5-en-3-ol,21-bromo-20-methyl-, (3.beta.)-	$C_{22}H_{36}O$	100
2	36.406	9,19-Cyclolanost-24-en-3-ol, (3.beta.)	$C_{30}H_{50}O$	95
3	34.609	Berbine, 13,13a-didehydro-9,10-dimethoxy-2,3-(methylenedioxy)-	$C_{20}H_{19}NO_5$	91
4	31.943	6H-Benzo[g]-1,3-benzodioxolo[5,6-a]quinolizine, 5,8,13,13a-tetrahydro-9,10-dimethoxy-, (.+/-.)-	$C_{20}H_{21}NO_4$	87
5	25.46	Cyclopentadecane	$C_{15}H_{30}$	77
6	26.200	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	79
7	20.173	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	71
8	20.253	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	72
9	20.556	Octadecanoic acid	$C_{18}H_{34}O_2$	72
10	17.249	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	67
11	13.077	Benzoic acid, 4-hydroxy-3,5-dimethoxy-, hydrazide	$C_9H_{12}N_2O_4$	61
12	13.209	n-Decanoic acid	$C_{10}H_{20}O_2$	27
13	12.431	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	61
14	13.461	Nonane, 4-methyl-5-propyl-	$C_{13}H_{29}$	61
15	13.953	Heptanoic acid	$C_7H_{14}O_2$	63
16	11.607	8-Pentadecanol	$C_{15}H_{32}O$	59
17	13.369	Hexanoic acid, 6-bromo-	$C_6H_{11}BrO_2$	61
18	32.87	6H-Purin-6-one, 2-amino-1,7-dihydro-	$C_{10}H_{13}N_5O_5$	89

DPPH free radical scavenging effects

The antioxidant potential for essential oils of T.ammi was measured using DPPH scavenging assays. In DPPH inhibition, oil was immensely active, presenting 83±0.06% inhibition at 1000 µg/mL concentration, while at the same concentration of plant extract showed 68±0.08 % inhibition (Table-2).

Table-2: DPPH free radical scavenging effects of *T.ammi* crude methanolic extract Essential Oil.

Samples	DPPH scavenging at different Conc.(µg/ml)			
	100	500	1000	IC_{50}
Essential Oil	35 ±0.01	40±0.02	68±0.08	400 ± 0.05
Plant extract	40 ± 0.5	70 ± 0.06	83 ±0.06	275± 0.03
Ascorbic acid	60	75	100	80± 0.01

Our results exhibited a strong antioxidant effect of oil in the DPPH scavenging assay due to phenolic components like tocopherols, flavonoids, and phenolic acids, which are natural antioxidants in foodstuff as well as in the therapeutic world. *T. ammi* essential oil has attractive appreciation in the health field due to its reasonably harmless position and its defensive effects [12]. Cells are damaged because free radicals irritate chain reactions within the cell. Free radicals are produced due to the oxidation of biomolecules such as carbohydrates, lipids, nucleic acid, and protein, which cause infection.

These free radicals scavenge by antioxidants, so the cells are protected from injury. In varieties of fruits and seeds, Phenolic compounds show antioxidant activities.

T.ammi is frequently supposed to be safe for treating digestive tract disorders and inflammatory diseases for a long duration of time [13].

Antibacterial effect

The antibacterial effect of essential oil extracted from *T.ammi* has been observed in Table-3. Results showed that *T. ammi* essential oil exhibited potent antibacterial action against the *E.Coli* (89%), *B. subtilis* (85%), and *S. aureus* (80%), respectively. The crude extract also showed good antibacterial action against *B. subtilis* (89%), *S. aureus* (85%), and *E. Coli* (86%)) respectively. Many monoterpenoid phenolic compounds of ajwain oil show an inhibitory role in Antibacterial Activity [14]. Thymol and carvacrol antimicrobial action is due to injury in membrane reliability with change in the equilibrium of inorganic ions and P.H. hemostasis. Bacteria resistant to thymol inhibited the antibiotics, and bacterial pathogens of resistant [15]. The *T. ammi* Essential Oil and plant extract showed excellent antibacterial action due to different phytochemicals. The small amounts of formulations of such essential oils have been recognized to prevent the production of *E. coli* and *Streptococcus* typhae; that's why they have effectively been formulated into skincare creams. Our results showed that the essential oil was active in inhibiting the pathogenic bacteria with the possible mechanism of different activities. From plants, natural stabilizers are chemical mediators that stop the decay of products [16]. Chemical conformation presence of thymol and its various derivatives as crucial components. Due to the fact of high phenolic components like thymol and carvacrol, ajwain oil showed the best antimicrobial actions [17]. This antimicrobial action of thymol and carvacrol is due to the destruction of membrane integrity by the balance of inorganic ions and change in hemostasis P.H. P-cymene raises the antimicrobial effect of thymol or carvacrol. However, it has no antimicrobial action [18, 19].

Antifungal activity

Antifungal activity of essential oil and crude was done by following standard procedure. Results showed that the oil has a potent antifungal effect against both the *Species,* i.e., against A. *Flavus* (86%) and *A. Niger* (87%), respectively Table-4. The plant extract also showed an excellent antifungal effect against *A. Flavus* (82%) and *A.Niger* (85%), respectively. For the essential oils, fungi are mainly challenging targets to inhibit at macro and micro levels. The pathogenic fungi are dangerous to a large population and responsible for severe infections, particularly in people with immune deficiencies. Essential oils obtained from various herbs or plants showed decisive antifungal actions [20, 21]. Like other phytochemicals, essential oils might reduce the development of bio-film and microbial growth by definite mechanisms [22]. Antifungal action of *T. ammi* Seeds on fungal growths was found to inhibit the development of examined increases at 72-90 % [23].

Table-3: Antibacterial effect of *T.ammi* Essential Oil and plant extract:

Bacteria	Control	Zone of Inhibition (mm)		%age Inhibition	
	Erythromycin	Oil sample		Plant extract	
Escherichia *coli*	100	11	89	14	86
Bacillus subtilis	100	15	85	11	89
Streptococcus aureus	100	20	80	15	85

Table-4: Antifungal Activity of *T, ammi* Essential Oil and Plant extract.

Fungi	Control Terbinofine	Zone of Inhibition (mm) Plant extract		% Inhibition Oil sample	
		mm	% Inh	mm	% Inh
A.*flavus*	100	18	82	14	86
A.*niger*	100	15	85	13	87

Table-5: Urease Inhibition of *T, ammi* E.O and Plant Extract (IC_{50}).

Enzymes	Plant Extract	E.O	Thiourea
Jack bean Urease	35.56± 0.04	67.45±0.8	19.13± 0.1
Bacillus Pasterurri Urease	55.77±0.01	85.35±0.06	29.82±0.01

Urease Inhibition Activity

The inhibition of urease activity is considered a hopeful remedy for ulcers because it prevents *H.pylori* infection. Urease inhibitors have been reported to decrease ecological difficulties [8]. Essential oil of *T.ammi* and plant extract was tested for urease inhibition activity (Table-5) for additional comparison. Oil showed potent inhibition against B.P Urease and also showed inhibition against (*J.B Urease*), IC_{50} values of 85± 0.06 and 67.45 ± 0.8, respectively. Plant crude showed optimal results for B.P urease and Jack bean urease with IC_{50} values of 55.77± 0.01 and 35.56±0.04, respectively. The components of *T. ammi* seeds showed evidence for therapeutic uses in various disorders [24].

Conclusion

Essential Oil of *T. ammi* exhibited potent antioxidant free radical scavenging assay due to the presence of phenolic complexes like tocopherols, flavonoids, and phenolic acids, which are natural antioxidants in foodstuff well as in therapeutic remedies. *T.ammi* oil showed good antibacterial against *E.Coli*, *B. Subtilis*, and antifungal against *A. flavu*s and *A. niger* due to thymol and caracole antimicrobial action. Crude also showed strong antibacterial, and antifungal activity Elements of essential oils might show antifungal activity because of their accumulation in the molecules of lipophilic hydro-carbon of the lipid bilayer cell. Inhibition of Essential Oil against J.B and B.P urease offers an immune for many therapeutic uses of this remedial plant in many ailments.

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