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STUDY OF ANTIMICROBIAL ACTIVITY AND COMPOSITION BY GC/MS SPECTROSCOPIC ANALYSIS OF THE ESSENTIAL OIL OF *THYMUS SERPHYLLUM*

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The steam distillation of Thymus serphyllum yielded 0.48% of the essential oil. GC/MS spectroscopic analysis resolved the oil into 39 (thirty nine) constituents. Out of which twenty eight components comprising 80% of the oil were tentatively identified and Thymol (53.33%) was the most abundant constituent of the oil. The antimicrobial activity of the essential oil was determined by using zone of inhibition method. The activity was found against seven G (+) and three G (-) test organisms. The minimum inhibitory concentration (MIC) for the tested microorganisms was found to be highly significant. All these values were indicative as bactericidal and not as bacteriostatic.

Thymus serphyllum Linn (N.O.Labiatae) commonly known as "Ban-Ajwain" is a small much branched and strongly scented shrub. It bears tiny purple coloured flowers (1, 2). It is considered to be very beneficial whether used as an article of food or as a medicament. Its volatile oil is good for inveterate cough and also as antiseptic, athelmintic and carminative (3). Due to these facts that this plant is very useful as indicated by these reports but very little information is available on the biological activity of the essential oil, there was a need to find out more about the potential of this plant as antimicrobial agent. The present study was, therefore, designed to find out the composition of the essential oil by GC/MS and to assess the potency of the oil on selected microorganisms. Although a lot of work has been reported in the literature by different workers on the chemical composition of the essential oil of *Thymus serphyllum* by the conventional methods and gas chromatography (4), yet it is the first time that the characterization of the essential oil has been carried out by GC/MS.

MATERIALS AND METHODS

Plant material. The whole shrubs were collected and a voucher sample has been deposited at the Dr. Sultan Ahmad Herbarium, Department of Botany, GC University, Lahore Pakistan. The plant parts were dried under shade and were reduced to powder in order to rupture maximum cell walls of oil glands. A weighed amount of powdered plat material (250 g) was subjected to steam distillation for several hours until the distillate was free from the oil. The distillate was collected and was extracted with diethylether. The ethereal extract was dried over anhydrous sodium sulphate and distilled the solvent to free the oil. The physico-chemical characteristics were determined according to the conventional method (5).

GC/MS spectral analysis. A GC/MS spectrum of the oil was determined on JOEL model JMS-A × 5050 H mass spectrometer (JOEL, Japan) combined with Hewlett Packard 5890 Gas Chromatograph (JOEL, Japan). Samples were injected on INCOT fused silica column, coated with SE-30 and Helium as carrier gas, split ratio 1:100, EI positive mode, electrical energy 70 ev., ionization current 200 μA, ionization source temperature 250°C, interface temperature 230°C, column temperature programmed at 60°C for 4 minutes with 6°C/min rise to 230°C. Data acquisition and reprocessing were performed by JOEL.JMA-DA 5500 system with MS-48 TK library search system (JOEL, Japan).

Antimicrobail activity: The micro-organism *E. coli, Salmonella typhae, Shigella ferarie, Bacillus magaterium, Bacillus subtilis, Lactobacillus acidophilis, Micrococcus leuteus, Stephylcoccus albus, Stephlococcus aureus* and

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Vibrio cholera were procured from nutrient agar slants. Stock solutions containing 50, 100, 150 and 200 μg/cm^3 were prepared in DMSO (Zone inhibition Method). Amoxil (Pfizer), streptomycin (Pfizer) and kanamycin (Pfizer) were use as PC (positive control) antibiotics. A concentration of 100 μg/cm^3 of the standard antibiotics prepared in DMSO were used for comparison. Antimicrobial activities of the essential oil on the growth of the microorganism were checked by agar well diffusion method (6, 7). Minimum inhibitory concentration (MIC) and Minimum bacterial concentration (MBC) were determined by the tube dilution method using nutrient broth (8).

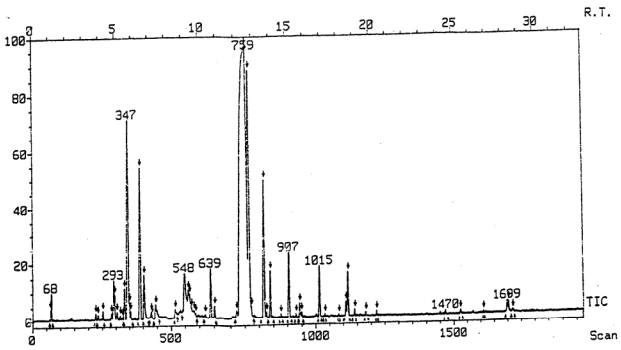
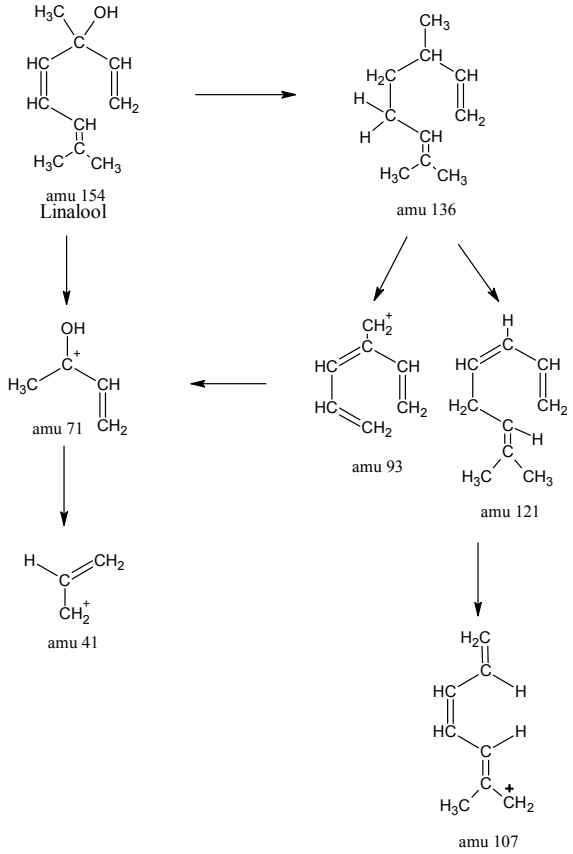


Figure 1. Total ions concentration of essential oil of *Thymus serpyllum* scan # 1 to 1971; RT 0'.00" to 32'.48"



Scheme A. Fragmentation and splitting pattern of various pecks of linalool.

RESULTS AND DISSCUSSION

The essential oil of *Thymus serphyllum* obtained in 0.48% yield showed the refractive index, specific gravity and optical rotation of the usual pattern of the essential as reported in the literature (9). The GC/MS analysis, the total ion chromatography (TIC) represented in figure 1, showed clearly the presence of thirty nine components in the essential oil. The identification of the components of the essential oil was performed by MS-48TK search system and was tabulated in table 1. As indicated, the resolved components were thirty nine, out of which twenty were tentatively identified where the seven components were in traces and twelve were not identified (table 1). It was recorded that the thymol (53.33%) was the most abundant component of the oil whereas carvacol (10.4%) was the second large component in abundance. Sur et al. (4) studied the composition of oils from three *thyme* species. Twenty components were identified from the oil of the aerial parts of *T. serphyllum*, *T. borystenicum and T. marshallianus* The major components reported in the oils were thymol and carvacol. Sattar et al. (5) studied the essential oil of some species of Labiatae family.

The composition of the essential oil of *Thymus serphyllum* reported had shown major components thymol (42.63%) and carvacol (8.16%) along with the other terpenes in minor amounts. It was worth while reporting that in order to establish the structure of a component, the splitting fragmentations were schematically designed to assign a structure to the terpenes. *Scheme A* showed the fragmentation and splitting pattern of various peaks of linalool. Similarly all the other component's structures were identified. The antibacterial activity of the essential oil was tested against ten bacterial stains in which seven were G (+) and three were G (-) at different concentrations of the essential oil and positive control. The results are shown in table-II, III and IV. The antimicrobial activity of the essential oil (100 μg/cm^3) found to be highly active except for *V. cholera* as compared to Streptomycin (100 μg/cm^3). The antimicrobial activity of the essential oil (150 μg/cm^3) found to be highly active except for *V. cholera* as compared to Streptomycin (100 μg/cm^3), however this concentration was less active for *S. typhea* against streptomycin (100 μg/cm^3) and for *M. leuteus* against Kanamycine. However for the essential oil concentration (200 μg/cm^3), the

Table 1. Spectral data of components of the essential oil of *Thymus Serpyllum* and component identified

	Retention Time	Compound (%)	Components Identified	m/e M^+ Fragmentation
01	1′4″	0.35	α-Pinene	93(100), 77(23.6), 136(16.4), 121(15.3), 105(10.7), 41(11.6), 67(7.8)
02	1′7″	0.24	Myrcene	93(100), 41(91.6), 69(76.7), 79(15.4), 136(12.6), 153(11.4), 107(4.8)
04	4′12″	0.2	Camphene	93(100), 121(82.4), 107(35.6), 79(34.8), 136(25.4), 39(23.4), 67(22.4)53 (15.6)
10	4′52″	1.0	d-Limonene	68(100), 93(65.80), 136(34.64), 121(25.4), 79(23.8), 107(20.75), 53(16.6), 41(15.4)
11	5′55″	0.3	β-Pinene	93(100), 41(58.1), 69(56.3), 79(13.4), 55(12.9), 136(11.2), 121(3.4)
12	5′3″	0.2	α-thujene	93(100), 77(60.3), 32(52.4), 136(26.8), 41(12.2), 105(9.8), 121(9.4), 65(8.6), 53(6.4)
15	5′23″	0.1	May be caryophyllene	
16	5′31″	0.9	α-Terepiene	121(100), 93(73.4), 136(47.2), 79(21.7), 105(14.5), 41(7.6), 65(7.4), 53(4.2)
17	5′46″	8.8	p-cymene	119(99.98), 134(100), 91(77.4), 77(25.6), 65(22.4), 103(18.4), 41(19.0), 51(11.2), 57(7.5)
18	5′53″	0.2	Terpinolene	93(100), 136(100), 121(86.4), 77(63.6), 43(35.5), 105(27.1), 35(14.2)53
19	6′30″	5.1	Δ^3 –Carene	93(100), 79(29.4), 136(23.6), 121(20.7), 41(3.4), 105(13.4), 67(8.4), 53(5.2)
20	6′43″	1.5	α-phellandrene	93(100), 69(29.6), 41(25.1), 79(20.4), 136(17.4), 121(15.3), 107(6.7), 53(5.8)
21	7′24″	0.5	Linalool	93(100), 71(89.2), 80(73.1), 121(63.3), 136(45.4), 107(20.4), 154(2.7)
22	8′33″	0.3	Fenchylalcohol	81(100), 93(61.92), 43(86), 55(52.25), 69(86), 111(66.69), 154(17.64), 139(56), 121(32), 43(66.6), 41(16.4)
23	9′7″	2.3	Borneol	95(100), 41(24.8), 110(22.2), 139(15.2), 81(11.9), 67(10.2), 121(7.9), 55(7.8), 32(7.5), 154(1.25)
25	10′37″	1.2	Terpin-4 ol	
27	12′37″	53.3	Thymol	
28	12′47″	10.4	Carvacol	
29	13′33″	4.9	Camphor	
32	15′5″	1.9	Terpineol	

biochemical behavior of the oil had been found to be very peculiar. It had shown very much low activity results for *S. ferarie* and *B. magnaterium* against amoxil, for *S. typhea, S. ferarie* and *Lactobacillus* against streptomycin and for *S. ferarie* and *V. cholea* against Kanamycine. The bacteria *S. ferarie* has shown immune behavior against the essential oil (150 μg/cm^3 and 200 μg/cm^3) but also against the positive control used. The antimicrobial behaviour of the essential oil at higher concentration had been very low activity for *S. ferarie, B. magnaterium, S. typhea, Lactobacillus, V. cholera* against the positive standards. It might be due to the immunity causing components be more in the essential oil at higher concentration, otherwise the essential oil had significant behavior at low concentrations. The results for the minimum inhibitory concentration (MIC) and minimum bacterostatic concentration (MBC) of the essential oil are presented in table V. the MIC refers to the minimum concentration of the oil with some inhibition. It was observed that the two-tailed T-test analysis at p=0.05 for the minimum inhibitory concentration (MIC) had been found to be highly significant. All these values showed the bactericidal activity of the essential oil and of the positive standards but not as bacteriostatic (10, 11).

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Table 2. Zones of inhibition (100 mcg/cm^3)

Microorganisms	Essential Oil	Negative Control (DMSO 100µL)	Positive Controls(100 mcg)		
	100 mcg/cm^3		Amoxil	Streptomycin	Kanamycin
Escherichia coli	38.50	-	19.50	28.50	21.25
Salmonella typhae	18.00	-	27.50	33.50	19.75
Shigella ferarie	17.00	-	25.00	28.50	25.50
Bacillus magaterium	24.00	-	12.65	34.00	32.75
Bacillus subtilis	22.30	-	15.25	15.75	19.00
Lacotbacillus acidophilis	19.20	-	14.00	31.50	22.25
Micrococcus luteus	18.20	-	21.00	20.75	27.50
Staphyllococcus albus	20.00	-	21.00	25.75	26.50
Staphyllococcus aureus	21.00	-	17.25	19.50	23.75
Vibrio cholera	18.00	-	25.00	18.75	26.25

Table 3. Zones of inhibition (150 mcg/cm^3)

Microorganisms	Essential Oil	Negative Control (DMSO 100µL)	Positive Controls(100 mcg)		
	150 mcg/cm^3		Amoxil	Streptomycin	Kanamycin
Escherichia coli	45.00	-	19.50	28.50	21.25
Salmonella typhae	24.00	-	27.50	33.50	19.75
Shigella ferarie	25.00	-	25.00	28.50	25.50
Bacillus magaterium	30.00	-	12.65	34.00	32.75
Bacillus subtilis	28.00	-	15.25	15.75	19.00
Lacotbacillus acidophilis	25.00	-	14.00	31.50	22.25
Micrococcus luteus	24.00	-	21.00	20.75	27.50
Staphyllococcus albus	30.00	-	21.00	25.75	26.50
Staphyllococcus aureus	30.00	-	17.25	19.50	23.75
Vibrio cholera	26.00	-	25.00	18.75	26.25

Table 4. Zones of inhibition (200 mcg/cm^3)

Microorganisms	Essential Oil	Negative Control (DMSO 100µL)	Positive Controls(100 mcg)		
	200 mcg/cm^3		Amoxil	Streptomycin	Kanamycin
Escherichia coli	52.00	-	19.50	28.50	21.25
Salmonella typhae	31.00	-	27.50	33.50	19.75
Shigella ferarie	33.50	-	25.00	28.50	25.50
Bacillus magaterium	38.00	-	12.65	34.00	32.75
Bacillus subtilis	37.00	-	15.25	15.75	19.00
Lacotbacillus acidophilis	33.00	-	14.00	31.50	22.25
Micrococcus luteus	31.00	-	21.00	20.75	27.50
Staphyllococcus albus	36.00	-	21.00	25.75	26.50
Staphyllococcus aureus	37.00	-	17.25	19.50	23.75
Vibrio cholera	32.00	-	25.00	18.75	26.25

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