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CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL OF *THYMUS VULGARIS* Linn. WHOLE PLANT

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ABSTRACT

This work was aimed at finding the chemical composition and antibacterial activity of the essential oil from the whole plant of Thymus vulgaris Linn. The essential oil was extracted using Clevenger's apparatus. Twenty four compounds were characterized by gas chromatography-mass spectroscopy (GC-MS). Thymol was characterized as the major constituent, followed by 1,2-benzenedicarboxylic acid among the volatile constituents. The antibacterial activity of the oil was tested against both Gram positive and Gram negative bacteria, Employing standard bacterial strains and Clinical isolates. Well cut method was adopted and the oil was found to inhibit Staphylococcus aureus ATCC 25923 and four clinical isolates viz., Proteus mirabilis from pus, methicillin resistant Staphylococcus aureus from throat swab, methicillin sensitive Staphylococcus aureus from pus and Escherichia coli from sputum.

KEYWORDS: *Essential oil, Gas chromatography-mass spectroscopy, Thymus vulgaris, antibacterial, well cut method, Clevenger's apparatus.*

INTODUCTION

Essential oils are known to posses many biological activities like wound healing[1], skin toning, antioxidant[2], antidepressant[3], as cosmetic[4]. *Thymus vulgaris* Linn. (Labiatae) is known for its essential oil content. *T.vulgaris* is a low, perennial under shrub, 20-30 cm high, found in the hills at higher elevations. It is extensively cultivated in Germany, France, England, Greece and various other countries, both for seasoning and for its volatile oil. The herb has a pungent taste. It is said to possess antiseptic, anthelmintic, expectorant, carminative, diuretic, emmenogogue and sedative properties due to the presence of a volatile oil[5]. The essential of *Thymus vulgaris* L. is known to be a powerful germicide and finds wide application as a disinfectant, antiseptic and of pleasant odor. It is used in many pharmaceutical and oral preparations like gargles and mouth washes. It is said to cause mental excitement. It is also used for flavoring all kinds of food products as condiment. Thymol is the most valuable and the main constituent of the oil. Carvacol is present in minor amounts. Amyl alcohol, *β*-pinene, camphene, *p*-cymene, *γ*-terpinene, linalool, borneol, geraniol, caryophyllene, sesquiterpenic alcohol. 4- terpineol, *trans*-4-thujanol were reported previously as the chemical constituents of the essential oil of *T. vulgaris*[6]. The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the number of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies[7]. One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents[8]. Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals, and plants. One of such resources is folk medicines. Systematic screening of them may result in the discovery of novel effective compounds[9]. The present study reports the analysis of the chemical constituents from the essential oil of *T.vulgaris,* whole plant, and its antibacterial activity.

MATERIALS AND METHODS

Plant material

The dried whole plant of *T. vulgaris* was collected from Regional Institute of Himalayan Flora, Tarikhet, India, was identified and authenticated by Dr.V.Chelladurai Ex. Research Officer (Botany) of SMPU unit of Central Council for Research in Ayurvedic sciences at Palayamkottai. A voucher specimen (no. 157A) was deposited in the museum of this institute.

Extraction of essential oil

Shade dried plant of *T.vulgaris* (100 g) was coarsely powdered and transferred in to a 1 lit round bottom flask. Sufficient amount of distilled water was added and fixed with Clevenger's apparatus[10]. This set up was boiled for 5 h and the steam-distilled essential oil was collected and dried over anhydrous sodium sulfate. The oil (0.28%) was transferred into an airtight sample tube and stored at 8°C.

Gas chromatography-mass spectroscopy analysis of the essential oil

Instrument

Shimadzu GC 2010 was the instrument used for gas chromatography (GC-MS) analysis. The constituents were identified by comparison of the mass fragments with the spectrum library NIST08s/WILEY8/FAME.

Procedure

One microliter of the essential oil of the plant was injected into GC. The injector temperature was maintained at 250°C. The detector used was flame ionization detector which was maintained at 280°C. The pressure of the carrier gas, nitrogen, was kept at 10 psi. The oven temperature was set at 60-280°C with a gradual increment of 10°C/min. The injected oil was eluted in the VF-5MS column of 30m length and 0.25 mm inner diameter and the eluted constituents were detected by flame ionization detector and the GC chromatogram was recorded.

Antibacterial activity of essential oil

The antibacterial activity of the essential oil was assayed using well cut method against both standard strains and clinical isolates.

Preparation of Inoculum

ATCC cultures were procured from National Chemical Laboratory, Pune and maintained by serial subculturing onto Nutrient Agar slants. The clinical isolates were procured from a NABL accreditated laboratory and used for testing. 2-3 colonies of the organisms were inoculated onto sterile nutrient broth and incubated at 37ºC for 24 hrs. The growth was indicated by turbidity. The standard strains that employed were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 700693, *Proteus vulgaris* ATCC 9484, *Proteus mirabilis* NCIM 2388, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, and *Bacillus cereus* NCIM 2106. The clinical isolates employed were *Escherichia coli* from sputum, *Staphylococcus aureus* from sputum, *Proteus mirabilis* from pus, methicillin sensitive *Staphylococcus aureus* from pus and methicillin resistant *Staphylococcus aureus* from sputum.

Well cut method[11]

The organisms were inoculated as lawn culture onto sterile Mueller Hinton Agar, 4 wells of 6mm were cut equidistant from each other. The essential oil was dissolved in dimethyl sulphoxide (250 µl in 500 µl). 10 µl and 20 µl of this was added into separate wells. Gentamicin (5 µg) was used as standard for Gram positive organism and norfloxacin (5 µg) was used as standard for Gram negative bacteria. The plates were incubated at 37ºC for 24hrs. Zone of inhibition was measured in millimeters.

RESULTS

The volatile oil constituents along with their retention time and percentage obtained from the GC-MS analyzer are given in Table 1. The chromatogram obtained is shown in the Figure 1. Twenty four constituents were identified in the volatile oil. The antibacterial activity was well expressed against most Gram positive bacteria tested and against *Proteus mirabilis* isolated from pus as given in Table 2.

Table 1. GC-MS data of essential oil of *T.vulgaris*

Compounds	Retention time (min.)	Area %
γ-Terpinene	5.355	3.89
β-Linalool	5.993	3.15
(-)-Camphor	6.843	1.70
4-Terpineol	7.312	1.87
(+)-*α*-Terpineol	7.536	0.29
2-Isopropyl-5-methyl-1-methoxybenzene	8.120	0.54
Bornyl acetate	8.816	0.98
Thymol	8.924	65.68
Isothymol	9.043	4.64
4-Ethyl-2,6-xylenol	9.643	0.11
(-)-*α*-Copaene	10.080	0.13
1-Cyclopropyl-3,4-dimethyloxyeugenol	10.394	0.33
Trans-*β*-Caryophyllene	10.704	4.50
1,3,6-Octatriene	11.176	0.08
2,6-Octadien-1-ol	11.226	0.12
6α-Cadina-4,9dine	11.391	0.36
Azulene	11.624	0.04
α-Cadinene	11.689	0.08
β-Bisabolen	11.758	0.65
1,6-Cyclodecadiene	11.886	0.39
β-Cadinene	11.926	0.73
Caryophyllene oxide	12.800	0.66
4-Butoxy-N-(4-methoxybenzyl) benzenesulfonamide	16.093	0.25
1,2-Benzenedicarboxylic acid	21.756	8.83

DISCUSSION

24 constituents identified, the percentage content of thymol and 1,2-benzenedicarboxylic acid were observed to be 65.68 and 8.83%. Whereas the content of *γ*-terpinene, *β*-linalool, (-)-camphor, 4-terpineol, isothymol, *trans-β*-caryophyllene were found to be <5%. Other constituents were <1%. The essential oil isolated from *T.vulgaris* was

found to contain 24 volatile constituents, of which thymol and 1,2-benzenedicarboxylic acid were the major compounds.

The oil was found to have profound antibacterial property. The concentration of the oil employed (5µl and 10µl) was almost similar to the concentration of the standard employed (5µg). Of the ATCC strains tested *Staphylococcus aureus* was found to be more sensitive to the oil, followed by *Bacillus subtilis* and *Bacillus cereus*. The Gram negative organisms tested did not show any susceptibility to the oil.

The clinical isolated tested were *Escherichia coli* and *Stahylococcus aureus* from sputum, *Proteus mirabilis* and Methicillin sensitive *Staphylococcus aureus* from pus and Methicillin resistant *Staphylococcus aureus* from throat swab. The oil was found to exert more activity against these clinical isolates when compared with the standard drug tested.

The growth of all the strains of *Staphylococcus aureus* tested was found to be inhibited by the oil. *Staphylococcus aureus* causes both superficial and deep pyogenic infections, as well as a number of toxin-mediated illness and important cause of urinary tract infections[12].

Investigation of its toxicity or irritant effects is required for using it as a topical medical agent. Hand disinfectants are an important means of reducing the spread of infectious microorganisms and the *in vitro* results from this study suggest that Thymus Oil could possibly be a useful topical agent for reducing staphylococcal loads on skin and the decolonization of nasal staphylococci.

Table 2. Antibacterial activity of essential oil of *T. vulgaris*

S.No.	Organisms	Zone of Inhibition		
		Standard	**Oil 10µl**	**Oil 20µl**
1.	*Escherichia coli* ATCC 25922	27	-	-
2.	*Pseudomonas aeruginosa* ATCC 27853	31	-	-
3.	*Klebsiella pneumonia* ATCC 700693	28	-	-
4.	*Proteus vulgaris* ATCC 9484	36	-	-
5.	*Proteus mirabilis* NCIM 2388	35	-	-
6.	*Bacillus subtilis* ATCC 6633	27	17	20
7.	*Staphylococcus aureus* ATCC 25923	29	20	24
8.	*Bacillus cereus* NCIM 2106	28	16	18
Clinical Isolates				
9.	*Escherichia coli* from sputum	30	-	-
10.	*Staphylococcus aureus* from sputum	25	16	22
11.	*Proteus mirabilis* from pus	21	20	22
12.	MS *Staphylococcus aureus*	21	20	24
13.	MR *Staphylococcus aureus*	18	18	21

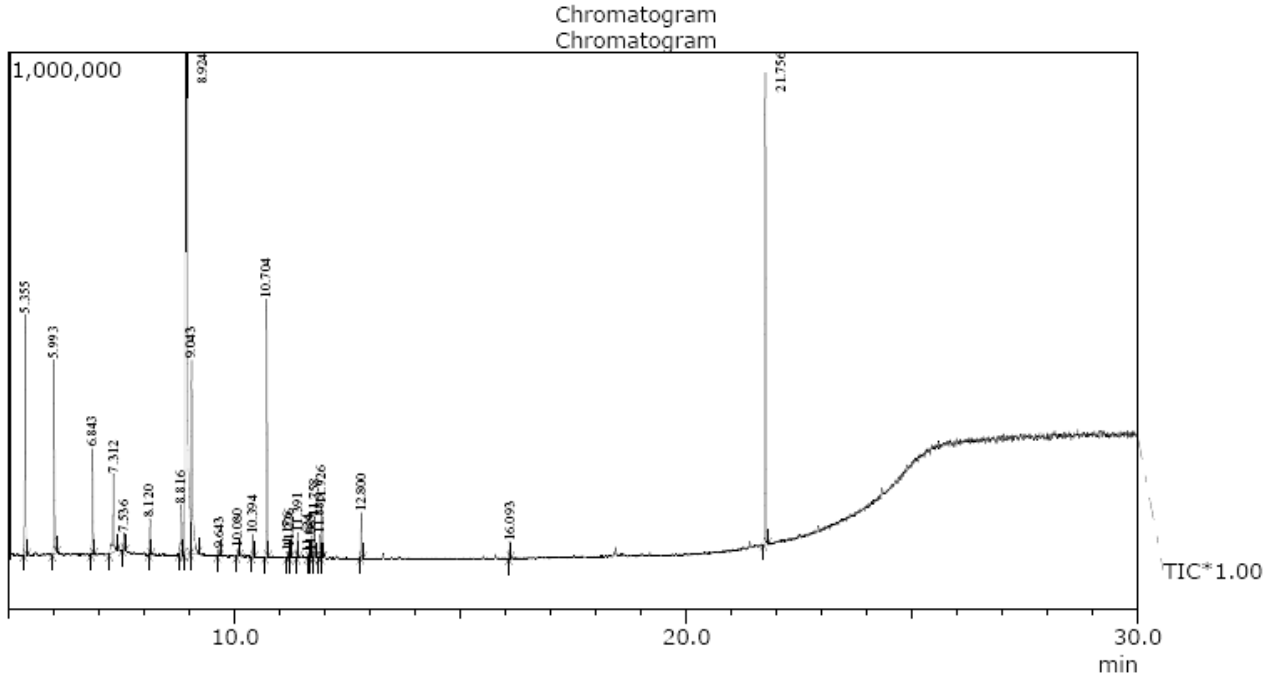

Figure:1 GC-MS Chromatogram of essential oil of *T. vulgaris* whole plant

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