e - ISSN - 2249-7722
Print ISSN - 2249-7730

International Journal of Phytotherapy

www.phytotherapyjournal.com

CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF THE LEAF OIL OF *THYMUS VULGARIS L.*

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ABSTRACT

The constituents of the leaf oil extracted by hydro distillation of *Thymus vulgaris L*, family, Lamiaceae from Ethiopia was examined by GC-MS. The yield of the yellowish oil was found to be be 0.97 % (w/w) in relation to dry weight basis. Eighteen chemical constituents were identified accounting for cent percent the leaf oil. The major terpenoids identified were thymol (31.977%), o-cymene (29.992%), carvacrol (14.541%), ç-terpinene (9.079%), linalyl anthranilate (4.762%), α-terpinene (1.756%) and 4-terpineol (1.464%). The leaf oil of *Thymus vulgaris* showed remarkable antibacterial activity against both gram negative (*Escherichia coli*) and gram positive (*Staphylococcus aureus*) bacteria.

Key words: Hydro distillation, *Thymus vulgaris L*, Lamiaceae, Essential oil, Antibacterial activity.

INTRODUCTION

Thymus vulgaris L, commonly known as Thyme, family, Lamiaceae is an aromatic perennial sub herb growing 15 to 30 centimetres high. It is native to Europe and western Mediterranean regions. Thyme oil is used as an aromatic additive in food, pharmaceuticals and cosmetics [1,2.] It possesses antiseptic, carminative, antimicrobial and antioxidant properties [3]. Thyme oil is also applied to reduce coughing, chest pain and to stimulate the production of saliva [4-7]. The aim of the present work is to investigate the constituents and antibacterial activity of the leaf oil of *Thymus vulgaris L* collected from Mekelle, Ethiopia.

MATERIALS AND METHODS

Plant material

Fresh Thyme leaves were collected from the growing field of Tarmaber woreda, in Amhara region, about 585 km far from Mekelle city and 198 km from the capital city, Addis Ababa, Ethiopia in January 2013. The plant first was identified by Mr. Hayal Lema, the botanist of Mekelle University. A voucher specimen of the plant was deposited at the National Herbarium, Department of Biology, Addis Ababa University with voucher number 088679.

Essential oil extraction

The shade dried leaves of *Thymus vulgaris L* plant collected (1Kg) was subjected to hydro distillation in a Clevenger apparatus for 3hrs. The essential oil was separated from aqueous layer using a 100 mL capacity separator funnel. The collected essential oil was dried over anhydrous sodium sulfate and filtered using a Whatman filter paper no. 40. The yield of the yellowish pleasant smelling oil was found to be 0.97 % (w/w) in relation to dry weight basis. It was stored in refrigerator at 4°C in dark brown 5-mL capacity sample bottle until analysis.

GC and GC-MS analysis

GC analyses were carried out in Agilent Technology 6890N gas chromatograph data handling system equipped with a spilt- split less injector using N_2 as carrier gas. The column was HP-5 capillary column

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(30m x0.32mm, 0.25µm film thickness) and temperature program was used as follows: initial temperature of 60 ^{0}C (hold: 2 min) programmed at a rate of 3^{0}C/min to a final temperature of 220^{0}C (hold: 5 min). The temperature of injector was maintained at 210 ^{0}C.

The GC-MS was performed by Perkin Elmer Clarus 500 gas chromatograph equipped with a spilt-split less injector (split ratio 50:1) data handling system. The column was an Rtx®-5 capillary columns (60 mm x 0.32 mm, 0.25µm film thickness). Helium was used as carrier gas at a flow rate of 1.0 mL/min. The GC was interfaced with Perkin Elmer 500 mass detector operating in EI+ mode. The mass spectra was recorded over 40-500 amu and revealed the Total Ion Current chromatograms. The temperature program remained the same as in GC. The temperatures of injector and transfer line were kept at 210 ^{0}C and that of ion source at 200 ^{0}C.

Identification of the oil components was done by comparison of their mass spectra with the NIST/Wiley library as well as by comparing them with those reported in literature. The identification of each compound was also confirmed by comparison of its retention index with those of authentic compounds [8].

Antibacterial activity

Preparation of test microorganisms

The test bacteria used in this study were: *Escherichia coli*, and *Staphylococcus aureus*. Both of the test bacteria were obtained from the College of Veterinary Medicine, Mekelle University, Ethiopia.

Media Preparation

Media was prepared by dissolving 38g of Mueller-Hinton Agar (MHA) in 1000mL of distilled water. Then heated completely to dissolve and it was sterilized using autoclave for 15minutes at 121^{o}C. 20 mL the sterilized media were poured into Petri dishes on a level, horizontal surface to give a uniform depth of media. The Petri dishes were covered and allowed to set at room temperature until the agar has solidified. Finally it was incubated at 37^{o}C for 24 hrs to be ready for susceptibility test [9,10].

Preparation of Agar disc diffusion method

The agar disc diffusion method was used to evaluate the antibacterial activity. Clinical isolates of *Escherichia coli*, and *Staphylococcus aureus* were obtained from the Department of Microbiology Laboratory, College of Veterinary Medicine. The stocks were maintained on nutrient agar slant and sub-culture in nutrient broth for incubation at 37^{o}C prior to each antimicrobial testing. Inoculation of the test organisms on nutrient agar prepared plates was achieved by flaming a wire loop on a spirit lamp, cooling the wire loop (air cooling) and fetching the test organisms. The standard inoculums suspension was streaked over the surface of the media using sterile cotton swab to ensure confluent growth of the organisms. 6mm diameter discs were prepared with Whatman No.1 paper and used for the study, and putting in vial-bottles and sterilizing in an oven at 150^{o}C for 15 minutes. Prepared discs containing the essential oil and positive controls were carefully placed on the inoculated plates using a sterilized forceps in each case [11]. The plates were then turned upside-down and inoculate at 37^{o}C for 24 hours in an incubator. After incubation, the inoculated plates were measured for zones of inhibition (in mm diameter by using electronic digital caliper). To standardize the inoculums density for a susceptibility test, 0.5 McFarland standards was used. A 0.5 McFarland standard was prepared as described in National Committee for Clinical Laboratory Standards (NCCLS) [12]. One percent v/v solution of sulfuric acid was prepared by adding 1mL of concentrated sulfuric acid to 99mL of water and mixed well. To make the turbidity standard, 0.5mL of the barium chloride solution will be added to 99.5mL sulfuric acid solution and mixed well. A small volume of those turbid solutions was transferred to a screw-capped tube of the same type as used for preparing the control inoculate and stored in the dark at room temperature. Amoxicillin (10UmL) and Tetracycline (30µg/disc) were used as positive controls and the discs were tested on the same microorganisms under the same conditions [12].

RESULTS AND DISCUSSION

The chemical composition of essential oil of Thyme is given in the table-1 and its GC-MS profile (fig-1) showed eighteen components in order of the retention times of the constituents. All the compounds in the leaf oil were identified. The yield of the essential oil contents observed in shade dried leaves of *Thymus vulgaris L* was 0.97 % (w/w). In the oil mixture, the percentage of monoterpenoids (93.707%) predominated over sesquiterpenoids (1.531 %).

The author's major monoterpenoids identified were thymol (31.977%), o-cymene (29.992%), carvacrol (14.541%), ç-terpinene (9.079%), linalyl anthranilate (4.762 %), α-terpinene (1.756%) and 4-terpineol (1.464%).

A report in the literature on the essential oil contents of *Thymus vulgaris L* showed the presence of thymol (44.4-58.1 %), p-cymene (9.1-28.5%), ά-terpinene (6.9-18.9%) and carvacrol (2.4-4.2%) as the major components [3]. But, Alessandra Zambonelli *et al* [13] found thymol (22-38%), γ-terpinene and p-cymene as the principal constituents.

An investigation by Shazia Shabnum *et al* [14] on the essential oil constitution of Thyme showed the presence of thirty components, among which thymol (46.21%), γ-terpinene (14.08%), p-cymene (9.91%), linalool (3.99%), myrcene (3.45%), α-pinene (2.97%), α-

thujene (2.84%) and carvacrol (2.44%) predominated in the mixture.

The eastern Morocco oil samples of thyme collected during the flowering period in the month of May showed an oil yield of 1.0% with camphor (38.54%), camphene (17.19%), α-pinene (9.35%), 1, 8-cineole (5.44%), borneol (4.91%) and γ-pinene (3.90%) as the major oil constituents [15]. The leaf oil of *Thymus vulgaris* showed a greater zone of inhibition against gram negative bacteria (*E.coli*) than gram positive bacteria (*S.aureus*).

Figure 1. GC-MS profile of *Thymus vulgaris L* leaf oil

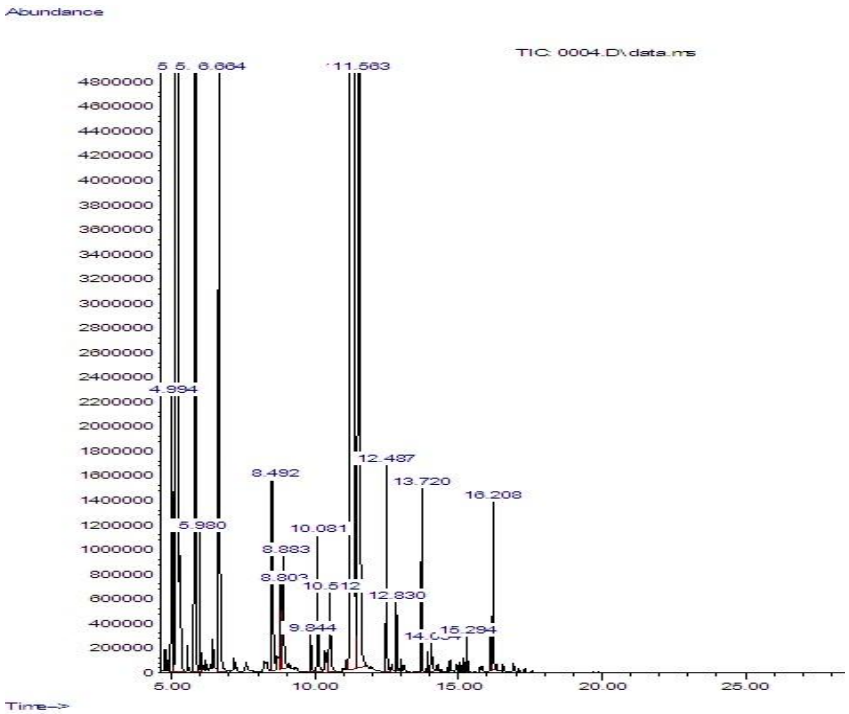

Table 1. Chemical components of *Thymus vulgaris L* essential oil

Peak No.	RT (min)	Identified compounds	% Compositions
1	4.994	α-Terpinene	1.756
2	5. 218	o-Cymene	29.992
3	5.839	ç-Terpinene	9.079
4	5.976	4-Terpineol acetate	0.564
5	6.660	Linalyl anthranilate	4.762
6	8.490	4-Terpineol	1.464
7	8.880	α-Terpeneol	1.400
8	9.848	2-isopropyl-5-methyl Anisole	0.206
9	10.079	2-isopropyl-4-methyl Anisole	0.698
10	10.511	α-Pinene	0.664
11	11.37l	Thymol	31.977
12	11.563	Carvacrol	14.541
13	12.489	Phenol,5-methyl-2-(1-methylethyl)-, acetate	0.882
14	12.825	Phenol,2-methyl-5-(1-methylethyl)-, acetate	0.265
15	13.720	Caryophyllene	0.722
16	14.057	4-(1,1-dimethylethyl)- 1,2-Benzenediol	0.219
17	15.294	Cedrene	0.160
18	16.208	Caryophyllene oxide	0.649
Total composition of identified components			**100.00**

RT = retention time

Table 2. *In vitro* antibacterial test of Thyme oil

Microorganism	Zone of inhibition	Amoxicillin	Tetracycline
E.coli	19mm	15 mm	25 mm
S.aureus	16mm	R	30 mm

R= Resistance

CONCLUSION

The author's report on the leaf oil contents of Thyme showed thymol (31.977%), o-cymene (29.992%), carvacrol (14.541%), ç-terpinene (9.079%), α-terpeneol (1.75%), and 4-terpeneol (1.464%) as the major monoterpenoid constituents. This report is almost in agreement with that of the analysis carried out by Baranauskine *et al* [3], Alessandra Zambonelli *et al* [13] but varies from other results.

The present report also showed the presence of linalyl anthranilate (4.762%) in the oil contents of Thyme. Laila Salim Al Hashmi *et al* [16] also reported the presence of linalyl anthranilate (1.064%) in n-hexane crude plant extract of *Thymus vulgaris* L along with other monoterpenoid components. These variations of the active principles present in the essential oils may be attributed to the different environmental and climatic conditions of different regions [17,18]. The report on more pronounced antibacterial activity of the Thyme leaf oil towards gram negative bacteria was supported by earlier researchers [19].

ACKNOWLEDGEMENT

The authors wish to thank the Dean, College of Natural and Computational Sciences, Mekelle University, Mekelle for providing laboratory facilities and the Test House, Banglore, India for furnishing the spectral information of the Thyme oil sample .

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