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Hydrodistillation extraction, GC-MS analysis and biological activities of *Thymus vulgaris* and *Mentha arvensis* essential oil

Saiqa Yousaf[1*], Sumera rani[1], Ashfaq Ahmad[1], Faiza Altaf[2]
[1] Department of Chemistry, Faculty of Science and Technology, Women University of Azad Jammu and Kashmir, Bagh, Pakistan
[2] Fatima Jinnah Women University Rawalpindi, Pakistan

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Abstract
The purpose of the study was to examine the chemical composition, antibacterial and antioxidant capabilities of Thymus vulgaris and Mentha arvensis essential oils. The essential oils were extracted using the traditional hydrodistillation process and evaluated using GC-MS. Thymus is a genus of valuable medicinal herbs that are highly recommended owing to the therapeutic effects of its essential oils, which are often known as thyme oil. The essential oils of Thymus vulgaris were tested for phytochemical activity and scientific support of traditional usage. Gas chromatography and mass spectrometry (GC-M) were used to examine the hydrodistillated oil produced from wild thyme species. Forty-seven components were identified. Among those thymol (60.55%), terpinene (8.53%), p-cymene (9.48%) and carvacrol (3.35%) were the major constituents. Other components were present in less than 2% of the total. Gas chromatography and mass spectrometry (GC-MS) were used to identify the components in Mentha arvensis essential oil. The essential oil study resulted in the discovery of twenty-eight compounds, accounting for 92.85% of the oil compositions. Menthone (29.42 percent), Menthol (21.35 percent), Isomenthone (10.85 percent), Eucalyptol (6.95 percent), neo-Menthol (4.75 percent), cis-Piperitone oxide (3.63 percent), Linalool (2.22 percent), Thymol (1.64 percent), Dl-Limonene (1.48 percent), and -Phellandrene (3.22 percent) were just the major components. Thymus vulgaris was investigated for antimicrobial activity against seven common food-borne bacteria and fungi: Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium, Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis, and Candida albicans using disc diffusion method. Thyme oil showed positive results against each pathogen. Antibacterial activity of *Mentha arvensis* was analyzed against six bacteria: *Using the micro wells approach, Candida albicans (C. albicans), Pseudomonas aeruginosa (Ps. aeruginosa), Proteus mirabilis (P. mirabilis), Enterococcus faecium (E. faecium), Escherichia coli (E. coli), and Staphylococcus aureus (St. aureus) were employed*. Essential oil gave positive results against each bacterium. The essential oil of Mentha arvensis was tested for antifungal efficacy against Candida flexuosus. At all concentrations, essential oil proved effective against F. solani. The antioxidant activity of thyme essential oil was determined using the DPPH test. Results showed, antioxidant activity is directly proportional to phenolic content.

Keywords: hydrodistillation extraction, *Thymus vulgaris*, *Mentha arvensis* essential oil

Introduction

Essential oils are chemical byproducts of odoriferous essences, extracted from one wide range of plants**.** Essential oils are low boiling liquids, insoluble in natural solvent, greatly soluble in alcohol and ether (organic solvents). Essential oils have different chemical compounds. Stem and leaves of *Thyme* species have been commonly used. These species have been generally used in traditional teas, sauce and flavouring agents and many medical purposes. (Nikolic, M., *et al*., 2014) [10]

Essential oils are enlisted for their familiar properties, i.e. antiseptic, fungicidal and insect killing properties. Until now about 3000 herbal oils are observed, out of 3000, three hundred have commercial importance, i.e. for the medication, to manage fields, nutriment, antiseptic and cosmetics. Components of essential oils are used in scents, cosmetics, sanitary substances, to cure teeth problems, as food preservers and as natural cures. Components like d-carvone Scents, cosmetics, detergents, flavor enhancers, cleansers, and solvents using d-limonene or geranyl acetate are all made with it. Essential oils are used in lotions, but most commonly in the perfume industry. There are some essential oils which possess important medicinal characteristics which is proven to treat organ malfunction or other disorders. All cultures have used herbs and parts of plants as medicinal source. Today, about 81% of the world's population use natural sources as basic health cure necessities. The majority of conventional treatments rely on the progressive concept of plant extracts (F. Bakkali *et al*., 2008) [2].

Introduction of vital 0il of *Thymus vulgaris*

The genus *Thymus*, member of the Lamiaceae family, contains approximately 400 species as perennial odoriferous, semi-evergreen or evergreen blossoming plants. (De Martino *et al*., 2009) [2]

Thyme (*Thymus vulgaris*) belongs to family Lamiaceae is a fragrant and medically important plant having high economic worth for North Africa, Europe and North America.

This plant is currently grown on a big scale in Iran. Obviously, thyme plays an essential role in the increasing global industry. Thyme oil is been investigated from less than 1 percent to 5

percent. Thyme oil contains phenolic components e.g., Thymol and carvacrol. On another hand, linalool and p-cymene were major nonphenolic components. Thymol and carvacrol have recorded to react as antioxidizing agent, disinfectant, fungicidal, wound healer, relieves flatulence, laxative and expels intestinal worms.

Plant material products, essential oil composition, and metabolites in medicinal plants are substantially influenced by harvest time, ecological and climatic circumstances, genetic alterations, and other environmental variables such as soil type and nutrition. Knowledge of the variables that influence drug production and plant oil composition in medicinally important plants is lacking, despite the fact that these factors have a considerable role in drug production and plant oil composition in medicinally essential plants. The current study aims to examine the essential oil composition, total phenol level, and free scavenging activity of Thyme grown from seed. This study can deliver useful information for observing, interaction effects between medicinal plants origins and climatic conditions on essential oil in plants having medicinal value. (A. Alizadeh, *et al*., 2013) [4]. Over the last few years, there has been a surge of interest in natural substances collected since herbs as medicines, scents, make up products and flavouring agents. Due to different biological activities, essential oils obtained from odoriferous plants have gained high consideration. Essential oils constitution is highly effected due to inherent factors and environmental effects. Due to this cause, plants of the same specie but in different regions give different composition and properties. Vital oil of Thyme is been recorded to exhibit many biological characteristics. Antimicrobial activities of the essential oil of *Thymus vulgaris* and of its component are discussed in some papers. Radical scavenging feature of thyme makes its valuable for nutriment protection. (Mancini, E. *et al*., 2015) [5]

Thyme is an odoriferous plant, mainly used as condiments and as natural health cure. These are recorded for exhibiting a few biological properties such as antibacterial, antifungal and radical scavenging activities. Thyme is depicted by many species of which some are native. Plants at blossoming stage have mainly acids phenols, tannins, resin, flavonoids, and mainly chemical compounds present in essential oil are the reasons for the great amount of its medicinal properties. In folk medicines, Thyme have been using for the cure of diarrhea, delirium, coughing, and injuries. It has also restorative, refreshing and mainly anti-inflammation characteristics after immediate relevance and mouth treatment. (Jaafari, A. *et al*., 2007) [6]

Essential oils extracted from fresh leaves and flowers can be utilized as fragrance additives in dietary products, medicines and make up products. Basil is a medicinal plant used in the cure of headache, loose motions, skin allergy, coughs, constipation, and kidney dysfunction.

It has also many useful effects as disinfectant, flatulence relieving, antibacterial, antifungal and antioxidative characteristics. Thymol is the main component present in essential oil which is active against *Staphylococcus* and *Salmonella* bacteria. It relieves cough, gives comfort in thoracic pain and trigger formation of saliva. (Shabnum, S. *et al*., 2011) [7]

Thyme essential oil and the plant extracts have shown many pharmacological activities. There are also many non-medicinal uses of *Thyme* e.g., in the dietary products and perfume industries, used as kitchen element and as a food preserver. Different extraction methods give different quality and yield of oil. (Grigore, A. *et al*., 2010) [8]

Thyme is the most famous plant due to its medicinal uses. *Thyme* have powerful biological properties. Relationship between components of oil and biological activities is shown by studies. (Imelouane, B., *et al*., 2009) [9]

Thymus L. consists of about 214 to 351 species based on statistical analysis. These are normally small evergreen plants present in Europe, Asia, and Africa. Green parts are mainly used for making teas, as taste makers and for many medicinal purposes. Many traditional curing properties are attributed to extraction of water soluble drugs. *Thyme* oil also protects food from oxidation. *Thymus* species are most commonly used medicinal herbs. An everlasting herb *thymus vulgaris* is native in Asia, Africa and Europe. It is full of essential oils and Anti-oxidative phenolic substances are enriched in Thyme essential oil. It has use as traditional treatment for gastro enteric and respiratory problems, antiparasitic, for relieving flatulence, and anti-depressant. Thyme species are also used for treating pain and increase passing of urine. Compounds present in essential oil improve the immunity system. Its oil cures joints pain, and is effective for hair treatment. (Milos Nikolic *et al*., 2014) [10]

Thyme oil is a good preserver of food. It is usually used in folk medicines. This is proved to be active against many fungi viruses and bacteria. (kateryna kon, Mahendra rai., 2012).

Food-borne illnesses give a major public health problem. Polluted food consumption with parasites activate the growth of disease causing microorganisms. In addition, there is a high demand for common food preservation method. Essential oils have strong microbial resistance. So, constituents of essential oils are highly used in medicines, food products as taste makers and in cosmetics. (Miladi H. *et al*., 2013) [12].

A significant aromatic plant *Thymus vulgaris* L. have almost 100 species which are highly used in kitchen dishes as well as for medicinal benefits. Except five percent which is cultivated commercially, other species are wild. *Thymus vulgaris* L. is an evergreen herb native in Europe, Asia, America & Africa. It is traditionally used for the treatment of many issues such as gastro enteric and bronchopulmonary disorders**.** (Rustaiyan, A et at., 2000) [13].

Introduction of essential oil of *Mentha arvensis.*

Mentha arvensis is a Lamiaceae plant that is also known as Japanese mint, menthol mint, and maize mint. Mentha plants have leaves, flowers and underground creeping stems. Perfumes and taste maker industries also use essential oils extracted from different sources. It is also used as food flavouring agent. *M. arvensis* L. is also used in mouth wash, sweets and drinks. (Vivek Sharma *et al*., 2009) [14] Aromatic plants have used for dietary products, make up products and drug industries. Demand of natural products is increasing day by day despite of the fact many of them were replaced by synthetic products. Higher plants have secondary metabolites in a great configurational variety. There are metabolites which keep plants safe from microorganisms. The secondary metabolites also influence neighboring plants growth. Some constituents have sprout inhibitor and insecticidal activities. Aromatic compounds and terpenoids are present in major amount in essential oils. Hydrocarbons are nearly always present in monoterpenes. All parts of the plant may accumulate essential oils e.g., in leaves, woods, barks, flowers, rhizomes

fruit, and seeds. The effects of the climatic condition also affect the constituents of essential oils. One important issue is the ingestion of airborne microorganisms and associated endotoxins. Resistance against contagious problems among microorganisms has grown. This problem persuaded for investigating other antimicrobial agents from different sources having medicinal plants. Recently, a large-scale work on essential oils activities against disease causing microorganisms has been carried out. (Mickiene, R *et al*., 2011). *Mentha arvensis* which is an vital oil containing crop is cultivated for menthol due to its high use in medicines, make up products and taste maker industries. Mints was known into India from Japan in 1952. *Mentha arvensis* L. Leaves have many medicinal activities e.g., antimicrobial activities. Compounds with antibacterial activity are significant because the prevalence of infectious illnesses caused by antibiotic-resistant microbes is rising. From the last three decades, up till now, there has been an increasing attention in the investigation for natural products from plants. There are many plants which have synthesized phenolic compounds as active secondary metabolite for insecticidal and anti-microbial activities, which is useful in natural therapies pharmaceuticals and alternative medicines. Some work has recognized and separated the reactive component in the herbs and plants accountable for the antimicrobial propertiesAs a result, greater research into the utilisation of plants as medicinal agents, particularly those connected to microbial management, should be prioritised. Moreover, we observed coordinated effects of essential oil of *Mentha arvensis* L. with antimicrobic property against bacteria. (Sugandhi, B. R. M *et al*., 2011) [16]. The use of herbal products as oral care agents is an ancient tradition and practiced in many parts of the world. Plant treatments are increasingly being known by scientists as a very important substitute to industrially produced expensive antibiotics and side effects related with them. Mentha is a plant genus that encompasses 25 to 30 different species and is well-known for its antibacterial, antiviral, and insecticidal properties. Mint essential oils are widely utilised in the cosmetic, food, and pharmaceutical sectors due to their fragrant, stimulating, and flatulence-relieving properties. Mentha arvensis, often known as pudina (menthol mint, maize mint, or wild mint), is widely cultivated in Bangladesh, Nepal, India, Sri Lanka, Japan & Thailand. This is used in traditional medicine to control indigestion, peptic ulcer and skin diseases. Antibacterial action of extracts of *M. arvensis* was expressed on uropathogens and enteric pathogens by various investigators. (Ramanath Karicheri *et al*., 2016) [17]

Bacteria cause serious health problems. Many bacteria have developed resistance to drugs. World Health Organization (WHO) reported that folk medicines are massively used for the cure of many problems.

Plants, a big source of natural organic compounds and are majorly employed in the preparation of medicines. The essential oils isolated from the aromatic plants have medicinal importance against microbial compounds. Plants essential oil have much potential against disease causing microorganisms. Essential oils extracted from plants have many class of compounds e.g., ketones, carbohydrates, ethers, alcohols, phenols, aldehydes and phenols which are responsible for biological properties. Since old time, these plants have been using for medicinal purposes and food additives. Aromatic plants like Mentha species are mostly grown in Saudi Arabia and are utilised in traditional medicine and herbal drinks. Mentha arvensis is the world's second greatest producer of essential oil. Mentha has a wide range of biological activity. Mentha arvensis essential oil is used as a carminative and nasal decongestant, for skin diseases and for the treatment of stomach. (Bukhari N. *et al*., 2016). Many plant products are used by Indians for the cure of human reproduction. *Mentha arvensis* L. have an anti-fertility property. Menthol is extracted from *Mentha arvensis* L. which is used in perfumery, food and pharmaceutical industries. India produces about 5000 tons of essential oil of mint. It has estimated that approximately 100000 hectares in India approximate produced 15000 tons of volatile oil during 1997. (Lohani H *et al*., 2012) [19].

Tropical and subtropical climates favor the production of *Mentha arvensis* L. Products and by-product of oil i.e., menthol and dementholized oil (DMO) have the major share in the world mint trade. (R.K. Sriwastra *et al*., 2002**)**

Objectives

- Extraction of oil from aromatic plants i.e., *Thymus vulgaris* and *Mentha arvensis*
- Essential oils analysis i.e., GC-MS
- To check antibacterial activity of essential oils
- To check antifungal activity
- To check anti-oxidant activity of essential oils
- To replace antibiotics with herbal medicine to isolate natural products containing antimicrobial agents from the plant.

Material and Methods

Plant material

Thymus vulgaris

Thyme roots were collected during the flowering season from Ganga Choti, a mountain near Bagh Azad Kashmir. Shuaib Ahmad, lecturer Botany Department of Women University Azad Jammu and Kashmir, Bagh, identified the plant species. The herbs were then shade dried for ten days packed in plastic bottles and kept in a cool and dark place for further processing.

Description of plant

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Family: Lamiaceae
Genus: *Thymus*
Order: Lamiales
Local name: *Chikkal*
Specie: *Thymus vulgaris*
Binomial name: *Thymus vulgaris*

Fig 1: *Thymus vulgaris*

Mentha arvensis

Fresh aerial parts of *Mentha arvensis* when the plant has enough foliage (before flowering stage) were collected from Muzaffarabad (Azad Kashmir), in May 2016. Genus of the plant *Mentha* was confirmed by taxonomist from Department of Botany Azad Jammu and Kashmir University, Muzaffarabad.

Description of plant

Kingdom: Plantae
Division: Magnoliophyta
Order: Lamiales
Family: Lamiaceae
Genus: *Mentha L.*
Class: Magnoliopsida
Specie: *M. arvensis.*
Local name: Pudina
Binomial name: *Mentha arvensis L.*

Fig 2: *Mentha arvensis*

Hydro Distillation Extraction

Saparation of oil from *Thymus vulgaris*

Hydro-distillation is used for the extraction of essential oils. Hydrodistillation was carried out using a laboratory heater. Five-liter container was used. Sixty grammes of dried thyme powder and 2.5 litres of clean water were placed into a hydrodistillation equipment and extracted for 2 hours, until no more oil was found. This procedure was done 500 times for 500 grammes of thyme powder. After each fraction, thyme oil was decanted and collected in Eppendorf tubes. Anhydrous sodium sulphate was used to dry the hydro distillation.

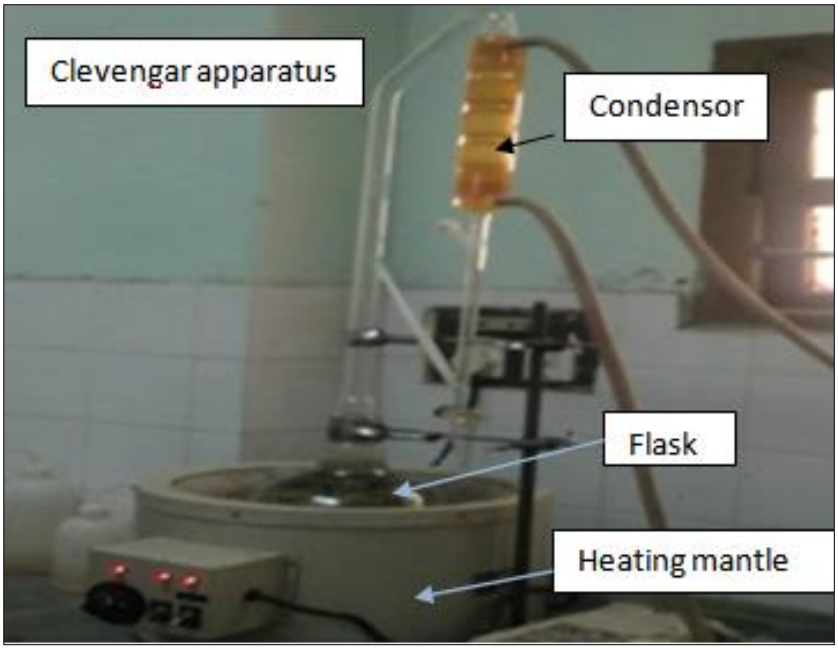

Fig 3: Clevenger-type apparatus

Oil extraction from *Mentha arvensis*

One kilogram of leaves of fresh sample were grinded and put into a hydro-distillation apparatus. Hydro-distillation was carried out using a laboratory heater. Five-liter container was used. One fourth part of container was filled with fresh aerial parts with 2.5 liter distilled water into hydro-distillation apparatus, and oil was obtained for 2 hours. This process was repeated for four times. After each fraction, mentha oil was decanted and collected in Eppendorf tubes. Anhydrous sodium sulphate was used to dry the essential oil.

GC-MS analysis

General protocol used for both samples (*Thymus vulgaris* + *Mentha arvensis*) is as under:

GC-MS data was observed by GC-MS on a model (7890A) attached with FID and narrow tube called capillary column of 0.25mm diameter, 0.25µm film thickness and 30m length. Temperature 60-230ºC at 5ºC/min, maintained at 60ºC and 230ºC for 3 and 4 minutes. Nitrogen was employed as a carrier gas (2ml per min). Plant essential oil was also seen using a Hewlett-Packard model 6890 at 75 eV ionisation energy and a DB-5 capillary tube with 0.25mm, 30m, and 0.25m film thickness. The M-S source temperature was 20oC, the sample injection volume was 3L, the split ratio was 1:60, the interface temperature was 230oC, and the mass scan ranged from 30-655 atomic mass units. Carrier gas used be there was Helium (1.5mL/min).

Fig 4: GC-MS

Identification of Components

Homologous *n*-alkanes series was used to analyze retention index for all volatile constituents. Wiley's New York mass spectral (MS) library was utilised to match component mass spectra with preserved library data.

Antimicrobial activity

Thymus vulgaris

Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, and Salmonella typhimurium. Shortly after, the tested microorganism suspension (106 cells/ml) was grown on solid medium plates (Sabouraud agar was used for fungi and Muller-Hinton agar was used for bacteria). The paper (Whatman No. 1, pore size 11m) was impregnated with essential oils in quantities of 5, 10, 15, and 20L before being placed on the prepared agar. Plates containing bacterial strains were cooked for 26 hours at 38°C and 47 hours at 32°C, respectively. Ciprofloxacin (25g/disk) and cephalexin (15g/disk) were employed as positive controls for bacterial strains, while fluconazole (15 g/disk) was utilised for fungi. The diameter of the inhibitory zone after incubation was measured in millimetres. Each test was done in triplicate on at least three separate experiments.

Mentha arvensis

Antibacterial activity

The specific bacterial cultures: yeast *Candida albicans (C. albicans), Proteus mirabilis (P. mirabilis*), *Pseudomonas aeruginosa (Ps. aeruginosa), Enterococcus faecium (E. faecium), Escherichia coli* (*E. coli),* and the *Staphylococcus aureus* (*St. aureus*) all were used in this study. These five bacteria are frequently employed in the study of disinfection agents. The MIC (minimum inhibitory concentration) was determined using a broth dilution experiment. All experiments were carried out in 0.2 percent sterile peptone water. The bacteria were suspended in 0.2 percent sterile peptone water, with a visual turbidity of 0.6 Mc Farland (approximately 110 CFU mL-1). The oils were diluted in 0.2 percent sterile peptone water at concentrations ranging from 0.09 to 50.1 percent. Microwells were inoculated with standardised microbe suspensions. 50 l of microbe suspension and 50 l of essential oil (concentrations ranging from 0.09 to 50.1%) were poured into separate holes and heated at 38 °C for 26 hours. The essential oil solution of microorganism-peptone water was then dissolved in 1 ml tubes with 0.02 percent Tween 80. The bacteria E. coli Castellani, E. faecium Schleifer, St. aureus Rosenbach and Kilpper-Bälz, and Pr. mirabilis Hauser and Chalmers were grown on Blood Agar Base (Oxoid, UK) for 24 hours at 37 °C. Aeruginosa Migula was grown on Blood Agar Base N. 2 for 26 hours at 32 °C. Candida albicans Berkhout was grown on Malt Extract-Agar for 48 hours at 27 °C.

Antifungal activity

Fungal pure culture was poured into petri plates having PDA media for incubation. Inoculum was taken after seven days from the culture for further processing. Poisoned food technique was used for toxicity measurement. The concentration of each oil was 0.5ml on pre-sterile petri plates with 10 millilitres of PDA culture. In the control set, the essential oil was replaced with an equivalent amount of acetone (75 percent), and the raw extract was replaced with an equal amount of filtered water. The inoculum was taken on 5mm diameter disc from the 7 days growing culture of the microorganisms and put at the mid of each dish. Petri dishes were heated at 30°C for 6-7 days. At last, percentage for the inhibition of mycelial spread for fungi was measured one by one.

Fig 5: *Fusarium saloni*

Total phenolic content

Colorimetric method was used to check percentage of phenolic substances. As a reference, Gallic acid solution was tested.

Anti-oxidant activity

The essential oil of *Thymus vulgaris* was checked for 2, 2-diphenyl-1-picrylhydrazyl anti-oxidant property. In brief, five millilitres of DPPH solution were incubated with four different doses of extract combined with methanol. Spectrophotometer was being used to measure absorbance at 517 nanometers after 35 minutes of incubation. Reading noting process was done three times. Methanol was utilised as the control ingredient, while Quercetin was employed as the reference compound. Fresh solutions were taken and prepared daily. Following formula was used for the calculation of percent inhibition: I percent inhibition

= [(A blank -A sample) / A blank] 100 A blank represents the absorbance of the control reaction, whereas A sample represents the absorbance of the DPPH solution containing the plant extract. IC50 = sample concentration required to scavenge 50% of DPPH.

Outcome and Discussion

Chemical formulation

Thymus vulgaris essential oil chemical composition

Thymus vulgaris hydro distillation yielded yellow oil. The chemical makeup of Thymus vulgaris essential oil is shown in Table 2. The essential oil of Thymus vulgaris included 47 identified components. Terpinolene (3.15 percent), p-cymene (8.53 percent), -Terpinene (9.48 percent), carvacrol (3.35 percent), and thymol were the components with the highest percentages (60.55 percent). Other components made up less than 2% of the total. Figure 6 depicts a strong association between -terpinene and p-cymene concentration and thymol and carvacrol content in Thymus vulgaris essential oil.

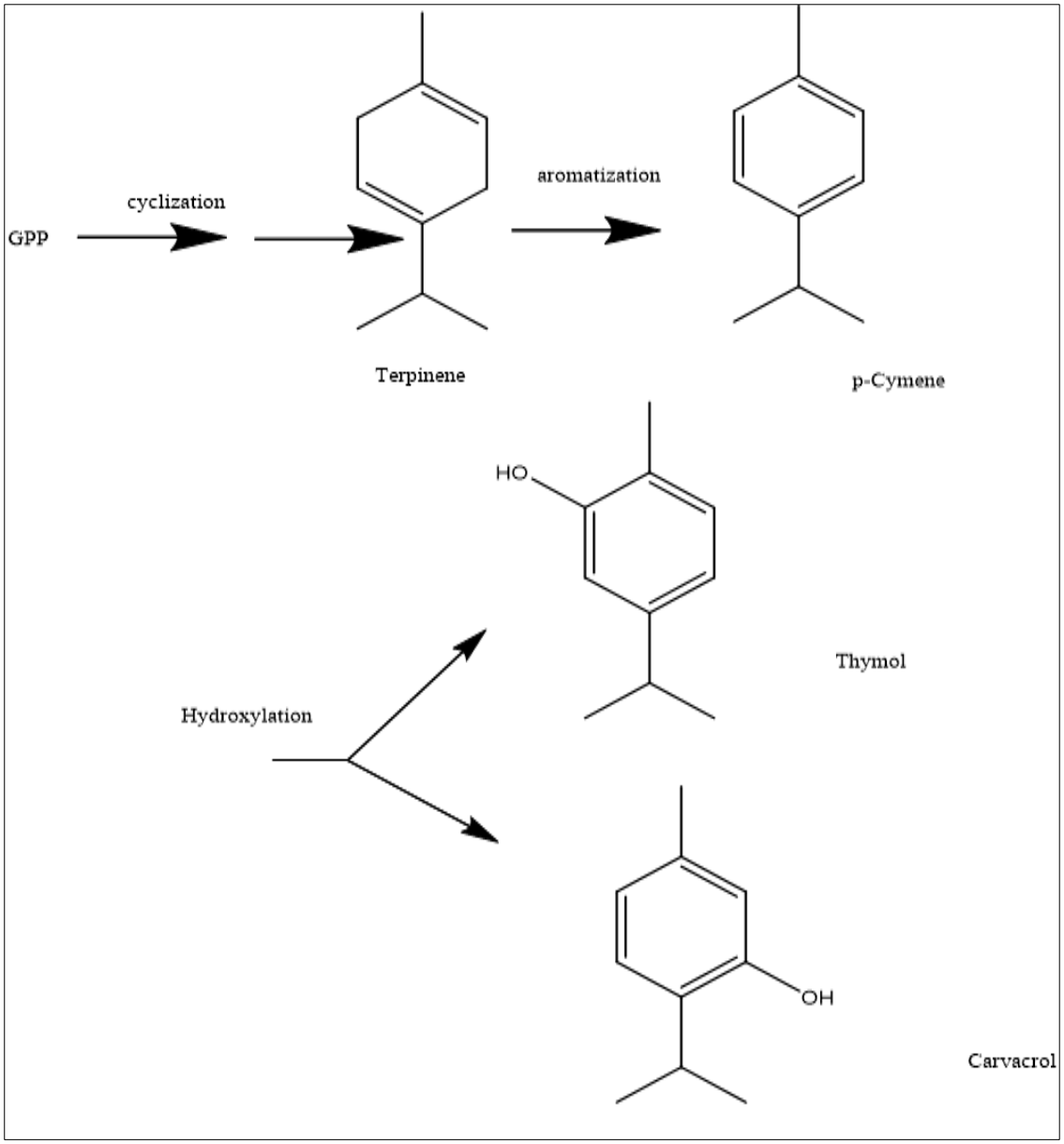

Fig 6: Thymol and carvacrol biosynthesis pathway

Table 1: *Thymus vulgaris* percentage

Plant	Fresh weight[a] (g/plant)	Dry weight (g/plant)	Essential oil yield[b] (%W)	Essential oil efficiency[c] (mg/ plant)
Th. Vulgaris	32.o ± 0.45	7.0 ± 0.15	1.5 ± 0.28	99.80 ± 0.35

These values are average of three experiments

In this case, "a" represents the dry weight in grammes. b is the oil yield given in milligrammes per 100 grammes of dry sample weight.

C is the oil's efficiency given in milli grammes per gramme of dry weight. 0.05 is the significance level.

Table 2: Essential oil compositions in *Thymus vulgaris*

No.	Compounds	RI[a]	*Thymus vulgaris*
1.	α-Thujene	929	0.86 ± 0.19
2.	α -Pinene	935	0.59 ± 0.14
3.	Camphene	950	0.53 ± 0.13
4.	Sabinene	975	0.04 ± 0.03
5.	1 -Octen-3-ol	981	0.24 ± 0.09
6.	3-Octanone	987	0.07 ± 0.03
7.	Myrcene	994	1.23 ± 0.03
8.	3 -Octanol	998	0.19 ± 0.03
9.	α -Phellandrene	1008	0.26 ± 0.13
10.	δ -3-Carene	1013	0.09 ± 0.03
11.	α- Terpinene	1019	1.25 ± 0.12
12.	P-Cymene	1026	8.55 ± 0.84
13.	Limonene	1034	0.09 ± 0.03
14.	1,8 -Cineole	1035	0.69 ± 0.16
15.	(Z)- *β*- Ocimene	1038	…….
16.	Benzene acetaldehyde	1045	……..
17.	(E) - β- Ocimene	1049	0.18 ± 0.05
18.	γ – Terpinene	1065	9.48 ± 1.84
19.	Cis-Sabinene hydrate	1070	0.85 ± 0.25
20.	Terpinolene	1090	3.14 ± 0.59
21.	Linalool	1105	0.10 ± 0.05
22.	Camphor	1145	0.13 ± 0.05
23.	Borneol	1167	1.26 ± 0.33
24.	Terpinene -4-ol	1179	0.23 ± 0.09
25.	γ – Terpineol	1210	0.26 ± 0.05
26.	Thymol methyl ether	1237	0.65 ± 0.15
27.	Carvacrol methyl ether	1245	0.40 ± 0.13
28.	Thymol	1301	60.55 ± 2.27
29.	Carvacrol	1315	3.36 ± 0.76
30.	Eugenol	1361	0.05 ± 0.03
31.	Isobornyl propionate	1379	0.37 ± 0.12
32.	*β*- Bourbonene	1387	0.09 ± 0.02
33.	(E) – Caryophyllene	1423	1.75 ± 0.24
34.	Aromadendrene	1440	0.09 ± 0.03
35.	α- Humulene	1455	0.15 ± 0.09
36.	Geranyl propanoate	1476	0.15 ± 0.08
37.	γ – Murolene	1478	0.25 ± 0.13
38.	Germacrene -D	1482	0.14 ± 0.05
39.	Valencene	1496	……..
40.	΄Ƴ- Cadinene	1515	0.20 ± 0.05
41.	δ- Cadinene	1525	0.24 ± 0.12
42.	α – Cadinene	1538	…….
43.	Spathulenol	1579	0.13 ± 0.05
44.	Caryophylene oxide	1584	0.35 ± 0.16
45.	10-epi-γ-Eudesmol	1620	0.13 ± 0.06
46.	EPI- α-Cadinol	1642	…….
47.	α-Cadinol	1655	0.19 ± 0.05
48.	Oil Yield (%w/w)		1.55 ± 1.27
	Total		99.5

RI[a] is the retention indices.
Values are means of three experiments ± SD.

Table 3: Thymol, P-Cymene, γ-Terpinene and Carvacrol contents of the essential oil in *Thymus vulgaris*

Plant	Thymol[a] (%)	P-Cymene[b] (%)	΄Ƴ-Terpinene[a] (%)	Carvacrol[a] (%)	Total[a] (%)
Thymus vulgaris	60.55 ± 2.28	8.53 ± 0.85	9.48 ± 1.85	3.35 ± 0.75	82.0

Values are the average of three experiments ± SD. $p < 0.05$.

Major components structures are as under:

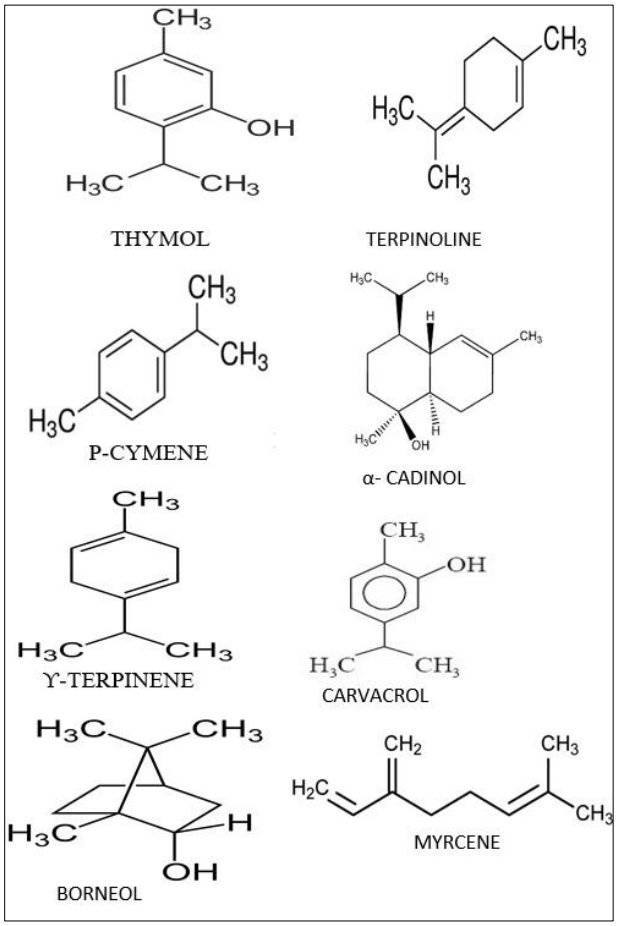

Fig 7

Mentha arvensis essential oil chemical composition

Hydro distillation was used to produce yellow oil. The components of the essential oil were identified using gas chromatography mass spectrometry (GC-MS). There were twenty-eight components found. -Phellandrene (3.22 percent), Dl-Limonene (1.48 percent), Linalool (2.22 percent), Linalool (2.22 percent), Cis-Piperitone oxide (3.63 percent), Menthone (29.42 percent), neo-Menthol (4.75 percent), Eucalyptol (6.95 percent), Isomenthone (10.85 percent), Menthol (21.35 percent), and Thymol (1.64 percent).

Table 4: Essential oil composition in *Mentha arvensis*

S. No.	RT[a]	Constituents	RI[b]	RI[c]	RA[d]
1.	3.872	dl-Limonene	……..	1155	1.48
2.	4.049	Eucalyptol	…….	1207	6.92
3.	4.459	α-Pinene	……	1040	0.69
4.	4.663	β-Pinene	……	1040	1.14
5.	4.789	δ-3-Carene	1202	1148	0.21
6.	5. 175	α-Phellandrene	1218	1217	3.22
7.	6.784	Octyl cyclobutane carboxylate	1284	……	0.33
8.	8.368	3-Octanol	1341	1383	1.83
9.	10.21	L-Menthone	1403	1458	29.42
10.	10.368	cis-Sabinene hydrate	1406	1522	0.70
11.	10.963	Isomenthone	1427	1454	3.84
12.	12.917	Linalool	1489	1540	2.22
13.	13.135	neo-Menthol acetate	1496	……	0.30
14.	13.875	trans-Caryophyllene	1519	……	0.52
15.	14.211	neo-Menthol	1530	1598	4.73
16.	14.343	4-Terpineol	1535	1553	0.30
17.	15.545	Menthol	1575	1614	21.35
18.	16.294	trans-Anethole	1598	1810	1.63

19.	16.446	δ-Terpineol	1603	1656	0.23
20.	17.138	2-Acetylfuran	1626	……..	1.37
21.	17.228	α-Terpineol	1627	1688	0.43
22.	17.384	cis-Piperitone oxide	1634	1701	3.63
23.	17.974	Isomenthone	1655	1453	6.99
24.	18.209	5-Isopropyl-6,7-epoxy-8-hydroxy-8-methylnon-2-One	1664	……	0.37
25.	22.752	2,6,6-Trimethyl-cyclohex-1-enecarboxylic acid	1670	……	0.42
26.	24.359	3-Methyl-3-(4-methyl-3-pentenyl) Oxiranemethanol	1876	……..	0.17
27.	24.587	Caryophyllene oxide	1885	1929	0.54
28.	27.784	2,5-Dimethyl-3-hexyne-2,5-diol	2000	…….	0.55

RT_a: Retention time.
RI_b: Retention indices on BP-20 polar column.
RI_c: Actual retention indices of columns (Supelcowax-10, HP-20M, CW-20M and BP-20).
RA_d: Components Percentage.
Some of the structures of major constituents are as under:

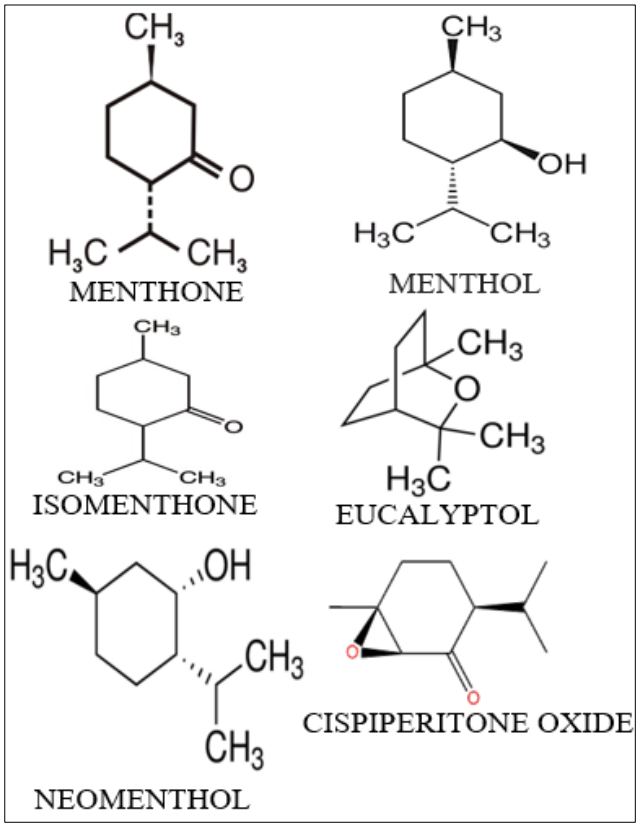

Fig 8

Antimicrobial activity

Antimicrobial activity of *Thymus vulgaris* essential oil

Table 5 shows the antibacterial efficacy of thyme oil against seven typical food-related bacteria and fungi. The null hypothesis that the inhibition zone is equal to the disc diameter (5 mm) was rejected for each microorganism at every amount of essential oil with a high significance level (p = 0.00). The main calculation of the ANOVA analysis is a strong interaction effect between the type of microorganism and the amount of essential oil (p = 0.00). The highly significant reaction effect adds difficulty in drawing general conclusions on the main effects, even if the two factors are also highly significant (p = 0.00). For example, *K. pneumoniae* has the highest inhibition zone overall but for amount of 20µL, where *E. coli* and *S. typhimurium* have higher values. To compare more thoroughly the effect of T. vulgaris on each microorganism Fig. 7 the results of multiple comparisons, at each oil amount, must be assumed. Tests revealed that the only microorganisms with non-significant differences in the antimicrobial effect are *S. typhimurium* and *E. coli* at all oil amounts, and *S. typhimurium, E. coli* and *C. albicans* at 10µL. The observed p-value for the pairwise differences in the above-mentioned cases does not pass appreciable significance levels, being larger than 0.35. All the other pairwise differences are highly significant i. e (p) = 0.00. K. pneumoniae, E. faecalis, S. aureus, P. aeruginosa, and E. coli growth suppression was previously documented, as was effectiveness against C. albicans and S. typhimurium. In contrast, several research show that thyme essential oil is ineffective against E. coli, S. aureus, and K. pneumoniae.

The inhibitions were measured in millimetres, and the paper disc had a diameter of five millimetres. The data distributions (n = 9) were reported as mean values and standard deviations (SD). As a

positive control, bacterial strains were given ciprofloxacin and cephalexin, while fungi were given fluconazole. The action of essential oils is determined by their chemical components. Essential oil action appears to be connected to phenolic chemicals (thymol) and terpene hydrocarbons (-terpinene). The third significant element component, p-cymene, does not exhibit antibacterial efficacy when employed alone; nevertheless, synergistic effects have been attributed to it in connection to thymol and -terpinene, respectively, which may represent another source of the antimicrobial activity seen. Several investigations, on the other hand, have demonstrated that essential oils have more antibacterial activity than their primary components or mixes, implying synergistic effects of the minor ingredients.

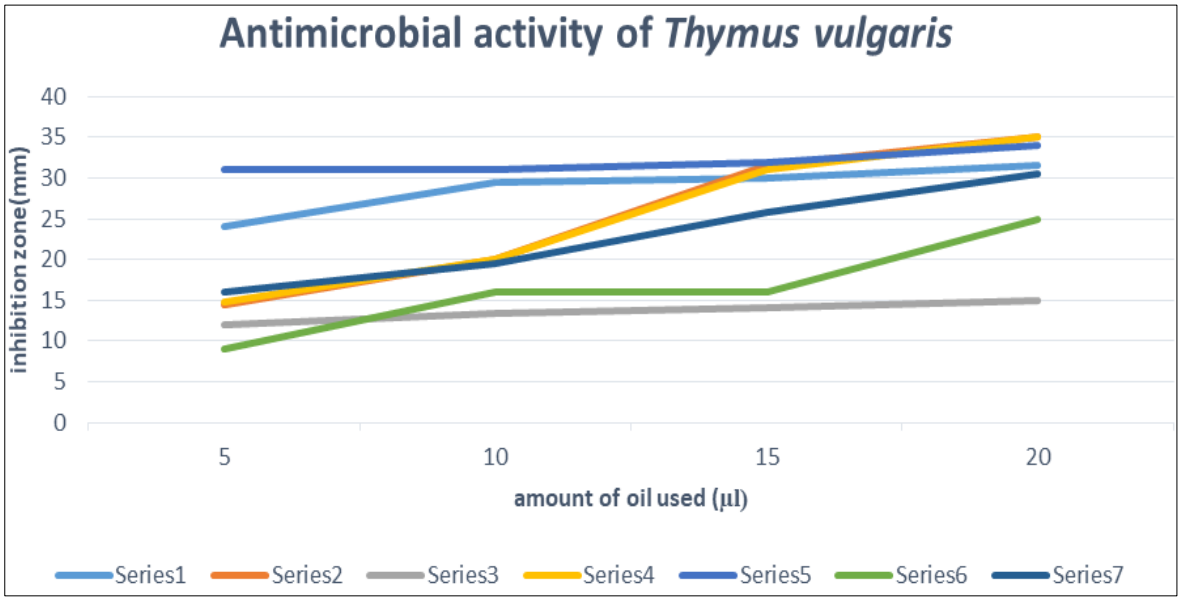

Fig 9: Essential oil antimicrobial activity of *Thymus vulgaris.*

Series 1: *Staphylococcus aureus*
***Series 2*:** *Salmonella typhimurium*
Series 3: *Pseudomonas aeruginosa*
Series 4: *E. coli*
Series 5: *Klebsiella pneumoniae*
Series 6: *Enterococcus faecalis*
Series 7: *Candida albicans*

Table 5: Antimicrobial activity of *Thymus vulgaris*

Test microorganism	Amount of essential oil (5µl)	Amount of essential oil (10µl)	Amount of essential oil (15µl)	Amount of essential oil (20µl)
Staphylococcus aureus	24 ± o.34	29.5 ± 0.7	30 ± 0.36	31.6 ± 0.48
Salmonella typhimurium	14.5 ± 0.35	20 ± 0.40	31.5 ± 0.35	35 ± 0.25
Pseudomonas aeruginosa	12 ± 0.27	13.35 ± 0.34	14 ± 0.23	15 ± 0.20
E. coli	14.7 ± 0.37	20 ± 0.42	31 ± 0.32	35 ± 0.20
Klebsiella pneumoniae	31 ± 0.13	31.05 ± 0.32	32 ± 0.25	33.98 ± 0.15
Enterococcus faecalis	9 ± 0.16	16 ± 0.16	16 ± 0.19	25 ± 0.16
Candida albicans	16 ± 0.39	19.5 ± 0.56	25.75 ± 0.25	30.5 ± 0.18

Antimicrobial activity of mentha arvensis essential oil
Antibacterial activity

The findings revealed a significant shift in the antibacterial activity of plant essential oils. Mentha arvensis oil demonstrated positive for antibacterial activity against six bacterial strains. The essential oil of Mentha arvensis had a strong antibacterial activity.

The results reveal that Mentha arvensis L. has significant antibacterial action against all microorganisms tested. Staphylococcus aureus and E. coli were both inhibited at a concentration of 3.0 percent. Minimum bactericidal dose of Mentha arvensis L. essential oil to prevent growth of all four bacteria, E. coli (5.8 5.7) ($p > 0.05$), E. faecium (50.7 17.8) ($p > 0.05$), St. aureus (1.20 1.8) ($p > 0.05$), and P. aeruginosa (38.8 5.7) ($p > 0.05$).

Mentha arvensis essential oil was used in our investigation. Mentha arvensis L. essential oil displayed significant activity against P. mirabilis, C. albicans, and a strong antimicrobial action against C. albicans, P. mirabilis, E. coli, and St. aureus, and only a minimal (0.9 percent) bactericidal concentration was required for these two germs. P. mirabilis (30.0 18.2) ($p > 0.05$) and C. albicans (69.67 32.5) ($p > 0.05$) were still developing at a concentration of 0.2 percent. Mentha arvensis L. essential oil had a minimal bactericidal concentration of 55.0 percent against four bacteria: Enterococcus faecium, Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli. and Staphylococcus aureus were substantially more susceptible to Mentha arvensis L. essential oil, requiring just a low (0.9 percent) bactericidal concentration. P. mirabilis (30.0 18.2) ($p > 0.05$) and C. albicans (69.67 32.5) ($p > 0.05$) were still developing at a concentration of 0.2 percent. Mentha arvensis L. essential oil had a minimal bactericidal concentration of 55.0 percent against four bacteria: Enterococcus faecium, Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli. Menthol from Mentha arvensis L. not only had sensory effects, but it also had

antibacterial and antifungal properties. Mentha arvensis L. is effective against both gram-negative and gram-positive bacteria.

Antifungal activity

Different plants oil showed different values Extract of *C. flexuosus* or F. Saloni showed fungal inhibition; 0%, 10%, 63.50%, 72.30%, 88.60%, 90.25%, of inhibition of fungal growth. 94.6%, 97.1%, 97.75%, 99% & 100% at 0, 0.7, 1.30, 2.7, 5.5, 10.6, 23, 32, 42, 55, 100 µl/ml concentration against *C. flexuosus* or *F. solani* respectively.MIC values of plants essential oils and extracts of F. Saloni or *C. flexuosus* were 05.5 & 50.5 µl/ml.

Table 6: Percent inhibition of *F. solani* growth on essential oil of *Mentha arvensis*

No.	Concent-ration of oil µlml^{-1}	Inoculum size (mm)	Colony size (mm)			Mean colony size (mm)	Mycelial growth (mm)	% inhibition of mycelial growth
			I	II	III			
1.	0	4	42	42	42	42	38	0
2.	0.7	4	19	18	19	18.67	14.67	10
3.	1.30	4	12	11	12	11.67	7.67	63.5
4.	2.7	4	8	7	8	7.67	3.67	72.30
5.	5.5	4	4	4	4	4.00	0.00	88.60
6.	10.6	4	4	4	4	4.00	0.00	90.25
7.	100.0	4	…	…..	….	……	…..	100.0

MIC = 5.5 µl/ml, mycelial inhibition: 0%, 10%, 63.5%, 72.30%, 88.60, 90.25 & 100% at 0, 0.7, 1.30, 2.7, 5.5, 10.6 & 100 µl/ml.

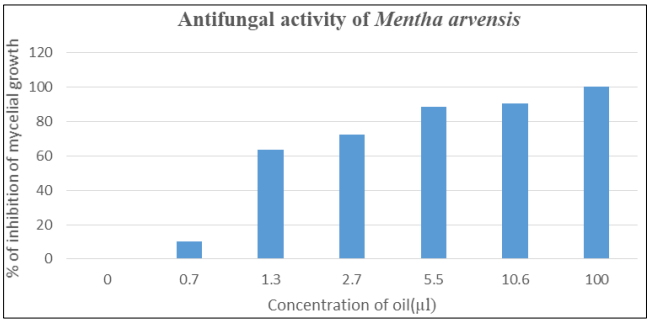

Fig 10: Antifungal activity of *Mentha arvensis*

Table 7: Antioxidant activity and Total phenolic content in *Thymus vulgaris*

Plant	Total phenolic content[a] (mg GAE/g DW.)	Antioxidant activity (IC50) [b] (µg/ml)
Thymus vulgaris	21.6 ± 0.12	7.8 ± 0.08

[a] Gallic acid (mg/g) dry weight (DW). [b] IC_{50}: µg/ml. $p<0.05$.
Values were average of three experiments ± SD.

Antioxidant activity and total phenolic content

Free radicals are the root cause of many health problems, including cancer and heart disease. These components, which can delay or prevent the oxidative loss of fats, lipids, or any other molecule due to free radicals, are likely to be protective against major health problems such as heart disease and tumours in animals and humans, and plant extracts containing antioxidants such as phenolic compounds may protect against free radical damage. Th. vulgaris had the highest overall phenolic content (21.6 mg GAE/g dry weight) (Table 8). The antioxidant DPPH was employed to test for free radical scavenging or oxidation. Table 8 shows the antioxidant activity of Th. vulgaris as measured by radical scavenging capability. Th. vulgaris has the greatest antioxidant activity (7.8g/ml). In Th. vulgaris, there was a link between radical scavenging ability and total phenolic content. The antioxidant action was mostly attributed to phenolic substances. According to our findings, increasing phenolic content resulted in an increase in antioxidant activity in Th. vulgaris. However, antioxidant activity in plants is not solely attributable to phenolic contents; it may also be attributed to the existence of other secondary metabolites such as vitamins, carotenoids, and volatile compounds. Thymus vulgaris has been found to be a powerful antioxidant or radical scavenger, and hence may be considered a natural antioxidant.

Summary

Essential oil composition of two plants, *Thymus vulgaris* & *Mentha arvensis* belonging to Lamiaceae family was analyzed through GC-MS analyses method. Hydrodistillation extraction method was used. Antimicrobial activities of two essential oils were also performed against pathogens. The antimicrobial activity of thyme oil against seven food poisoning fungus and bacteria was performed by applying disc diffusion procedure. Antibacterial and antifungal activities of *Mentha arvensis* was performed using micro wells method. Antibacterial activity of *Mentha arvensis* was checked against 6 pathogenic bacteria and antifungal activity was checked against *f. solani.* Anti-oxidant property of *Thymus vulgaris* essential oil was performed by using DPPH assay.

Forty-seven components were analyzed in GC-MS analysis of *Thymus vulgaris* while twenty-eight were analyzed in essential oil of *Mentha arvensis*. Most of the components were phenolics. Major components in Thyme essential oil were p- cymene, gamma terpinene, Thymol and carvacrol. Percentage of other constituents was less than 2%. Twenty-eight constituents were recognized in essential oil of Mentha arvensis. Menthone, Menthol, Isomenthone, Eucalyptol, Neo-menthol, Cis-Piperitone Oxide, and Linalool were the components with the highest percentages recorded. Others were less than 2%. Antimicrobial tests of Thymus vulgaris revealed that the only microorganisms with non-significant effects were S. typhimurium and E. coli at all oil amounts and S. typhimurium, E coli and C. albicans at 10 microliters.

Antibacterial activity of Mentha arvensis showed positive results against six pathogenic bacteria. Staphylococcus aureus and E. coli were both inhibited at a concentration of 3.0 percent. Mentha arvensis L. essential oil with menthol as the main component exhibited strong activity against P. mirabilis and C. albicans, as well as a strong antimicrobial property against C. albicans and P. mirabilis. St. aureus and E. coli were much more sensitive to the essential oil, and only a minimal (0.9 percent) bactericidal concentration was required for these two germs. Antifungal activity of essential oil of Mentha arvensis showed positive results for each concentration of oil. Antioxidant potential of Thymus vulgaris revealed that phenolic content and radical scavenging activity have direct relation. As both species belonging to Lamiaceae family have active components which showed active microbial activities, so, research should be carried on for further betterment to living beings.

Declarations
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