Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(1), 189-195 | ISSN:2251-6727

Gc/Ms Analysis of Thymus Vulgaris Essential Oil and Antibacterial Effect Towards Multidrug-Resistant Staphylococcus Aureus

Farah Abid[1,2], Mabrouka Bouacha[1,*], Ghania Bourzama[1], Lilia Boussouf[3,4], Hanane Boutennoun [4,5], Nafissa Boutefnouchet[6]

[1]Laboratory of Microbiology and Molecular Biology, Faculty of Sciences, University Badji Mokhtar, Annaba, Algeria

[2]Laboratory of Biochemistry and Environmental Toxicology, Faculty of Sciences, University Badji Mokhtar, Annaba, Algeria

[3] Department of Applied Microbiology and Food Sciences, Faculty of Nature and Life Sciences, University Jijel, 18000 Jijel, Algeria

[4] Laboratory of Biomathematics, Biophysics, Biochemistry and Scientometry, Faculty of Nature and Life Sciences, University Abderrahman Mira, 06000 Bejaia, Algeria

[5] Department of Molecular and cell biology, Faculty of Nature and Life Sciences, University Jijel, 18000 Jijel, Algeria

[6]Laboratory of Biochemistry and Microbiology, Faculty of Sciences, University Badji Mokhtar, Annaba, Algeria

(Received: 16 November 2024 Revised: 20 December 2024 Accepted: 04 January 2025)

KEYWORDS

antibacterial effect, Essential oil, GC/MS, *S.aureus*, *Thymus vulgaris*

ABSTRACT:

Introduction: Thymus vulgaris, often referred to as thyme, is a member of the Lamiaceae family, known for its many medicinal and culinary attributes. This plant is prevalent in hilly and semi-arid regions of North Africa, especially Algeria, where it grows naturally. In Algeria, thyme plays a significant role in traditional medicine and is often used in herbal teas, essential oils, and herbal formulations.

Objectives: This study aims to explore the chemical composition and the antibacterial effects of *Thymus vulgaris* essential oil from Algeria.

Methods: The essential oil was extracted via hydrodistillation and subsequently analyzed using Gas Chromatography-Mass Spectrometry (GC/MS) and the antibacterial effects of the essential oil was assessed using well diffusion and dilution assays against three standard strains and ten multidrug-resistant strains of *Staphylococcus aureus*.

Results: The results revealed that there are 28 different compounds in the *T. vulgaris* essential oil, the main compounds are thymol at 27.28%, phenol, 2-methyl-5-(1-methylethyl)- at 21.32%, and benzene, 1-methyl-2-(1-methylethyl)- at 20.2%. The results of the antibacterial effects demonstrated that *T. vulgaris* exhibits an excellent antibacterial effect with an inhibitory diameter ranging from 48.67±1.53 to 52.5±1.32 mm, MIC values from 1 to 2 µL/mL, and MBC values ranging from 1 to 4 µL/mL. The MBC/MIC ratio was always inferior to four, indicating a bactericidal effect rather than a bacteriostatic effect.

Conclusions: the essential oil exhibits a diversity of compositions with significant antibacterial effect against *S.aureus*. Therefore, *T. vulgaris* essential oil could be effective as an alternative antibacterial agent for the treatment various types of infections, especially those caused by *S.aureus*.

1. **Introduction :** *Staphylococcus aureus*, a Gram-positive bacterium, is frequent colonizer and one of the most opportunistic bacterial pathogens of humans [1]. It is a ubiquitous bacterium responsible for a wide range of

infections, from minor skin conditions such as abscesses and impetigo to severe and potentially life-threatening diseases such as pneumonia, endocarditis, osteomyelitis, sepsis, and toxic shock syndrome [2–4]. *S. aureus* is well known by its virulence, causing tissue necrosis and interfering with the immune system [5]. Penicillin initially revolutionized the treatment of severe *S. aureus* infections; however, resistance expanded quickly due to the acquisition of resistance genes, which is currently seen as a danger to global public health. The cornerstone of treatment for invasive MRSA infections hwas glycopeptides; however, decreased susceptibility due to adaptive mutations further hindered therapeutic efforts [1, 6]. The situation has become worse by the apparition of multidrug-resistant (MDR) *S. aureus*, which is resistant to numerous antibiotics. MDR *S. aureus* poses a significant difficulty in clinical settings [7, 8], severely limiting treatment options and leading to increased mortality, morbidity, and healthcare costs [9]. Its ability to spread easily in healthcare environments, form biofilms on medical devices, and continually evolve into new strains exacerbates the difficulty of managing infections [10]. MDR *S. aureus* utilizes various strategies to evade antibiotic therapy. A significant mechanism in this regard is genetic adaptability using genetic modification to change the antibiotic target sites [7]. Furthermore, MDR *S. aureus* may acquire resistance genes from other bacteria through bacteriophages, transposons, and plasmids [8] . It could also use efflux pumps to expel antibiotics from the bacterial cell, which lowers the intracellular concentration of antibiotics. Another critical strategy employed by MDR *S. aureus* is the formation of biofilms, a protective extracellular matrix, to increase resistance to antibiotics. These structures restrict antibiotic penetration and facilitate the exchange of resistance genes among bacteria [4, 8]. They also help MDR *S. aureus* evade the host immune system, persist on medical devices and tissues, and lead to chronic and recurrent infections [11, 12].

The World Health Organization (WHO) has repeatedly expressed alarm about the dangers of MDR strains and associated illnesses. This alarming situation has encouraged research in producing innovative and effective antimicrobial drug [13]. In this context, several novel treatment strategies, such as the use of essential oils, are being developed [14, 15]. Essential oils are secondary metabolites of plants, generally composed of terpenes, phenols, and aldehydes, which are efficient against many infections. Essential oils have antibacterial properties against drug-resistant microorganisms [16, 17]. Multiple factors are involved in the antibacterial actions of essential oils, which hinder the development of bacterial resistance [18–20]. Unlike antibiotics, which have specific target sites, essential oils are composed of varied chemical compositions. Each compound may have a unique mode of action and a unique target site, therefore, a synergistic effect could be produced, increasing the overall antibacterial activity of essential oils [16].

Numerous medicinal plants with considerable therapeutic potential are found in Algeria, a country renowned for its great biodiversity and diversified flora with 3000-3500 plant species, 15% of which are endemic [21, 22]. Various species have significant therapeutic properties, which makes them an important subject of scientific study, especially in the field of natural bioactive substances. The traditional medicinal use of Thymus vulgaris are particularly noteworthy. *T. vulgaris*, has been used for centuries for its aromatic and medicinal properties. Its essential oil, which has a high concentration of thymol, is well known for having potent antibacterial and antifungal properties [23].

2. Objectives

The chemical composition and the therapeutic effects of essential oil have been thoroughly investigated in the world. However, in Algeria, there is a notable lack of studies focusing on comparison of the chemical composition of these essential oils. Furthermore, few studies have been conducted on the antibacterial effects of several medicinal plants, including *T. vulgaris*. Therefore, the aim of this study is to compare the chemical composition and the antibacterial effectiveness of the essential oil obtained from T. vulgaris against standard and pathogenic *S.aureus* strains.

3. Methods

Medicinal plant essential oils

T. vulgaris were collected from the region of Sraïdi, Annaba, Algeria. The leaves were collected early in the morning, during the vegetative stage of the plant in April 2023. The region of Sraïdi is renowned for its mountainous terrain and unique ecosystem, particularly rich in wild natural medicinal plants. The abundance of wild natural medicinal plants, which have adapted to

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JCHR (2025) 15(1), 189-195 | ISSN:2251-6727

local conditions over centuries, has developed bioactive compounds for therapeutic purposes. The leaves were dried in the shade away from light at room temperature. 200 g of dried leaves were hydrodistilated for 3 hours using a Clevenger-type apparatus. The result was 1.2%(w/w) of oil. These were dried over anhydrous sodium sulfate and kept at 4°C until they were tested and analyzed.

Gas Chromatography–Mass Spectrometry analysis

The Schimadzu GCMSQP2010 model gas chromatograph was used to analyze the essential oils. It was connected to a quadruple mass spectrometer type EI model 70 eV that has a polar capillary column SE 30. Helium was used as the carrier gas at a flow rate of 1 mL/min, and the injection volume was 1 µL. The oven was set to begin at 60°C, retain that temperature for two minutes, and then rise to 240°C at a rate of three degrees Celsius each minute. With a scan range of 40–400 m/z, the MS detector was run in electron ionization mode at 70 eV. By contrasting, the compounds' mass spectra and retention indices with those found in the NIST database, the specific compounds present in the essential oils were accurately identified.

Antimicrobial effects

Thirteen bacterial strains of *S. aureus* were used to assess the antibacterial effects of the essential oils, including three standard strains and ten pathogenic strains. Bacterial identification was performed by conventional microbiology methods (Gram staining, oxidase, catalase, coagulase, and API STAPH). An antibiogram of the bacterial strains was performed by the disk diffusion method according to the Clinical and Laboratory Standards Institute [24] using Mueller Hinton agar medium (Difco, MD, USA). The following antibiotic discs was used : amoxicillin–clavulanic-acid (2/1 µg), oxacillin (1µL), ofloxacin (5µL), doxicycline (30 µg), fosfomycin (200 µg), colistin (10 µg), pristinamycin (15µL), sulfamethoxazole-trimethoprim (1.25/23.75 µg), clindamycin (2 µg), VA: vancomycin (5µL) (BioRad, France). Only bacteria that showed multidrug resistance were selected (Table 1).

Table 1. Susceptibility of the pathogenic bacteria to antibiotics.

Pathogenic bacteria	Type of infections	Susceptibility to antibiotics									
		AMC	OX	OF	DO	FO	CO	PR	SXT	CL	VA
S. aureus 1	Urinary infection	R	R	R	R	R	R	S	R	R	S
S. aureus 2	Urinary infection	R	R	S	R	S	R	R	R	R	S
S. aureus 3	Urinary infection	R	R	R	R	R	R	R	R	R	S
S. aureus 4	Skin infection	R	R	R	R	R	R	S	R	R	S
S. aureus 5	Skin infection	R	R	R	R	R	S	R	R	R	S
S. aureus 6	Skin infection	R	R	R	R	R	R	R	R	R	S
S. aureus 7	Respiratory Infection	R	R	R	S	R	R	R	R	R	S
S. aureus 8	Respiratory Infection	R	R	R	R	R	R	R	R	R	S
S. aureus 9	Respiratory Infection	R	R	R	R	R	R	S	S	S	S
S. aureus 10	Respiratory Infection	R	R	R	R	R	R	R	R	R	S
S. aureus ATCC 25923	/	S	S	S	S	S	S	S	S	S	S
S. aureus ATCC 29213	/	S	S	S	S	S	S	S	S	S	S
S. aureus ATCC 43300	/	R	R	S	S	S	S	S	S	S	S

AMC: amoxicillin–clavulanic-acid, OX: oxacillin, OF: ofloxacin, DO : Doxicycline, FO: fosfomycin, CO :colistin, PR : pristinamycin, SXT: sulfamethoxazole-trimethoprim, CL: clindamycin, VA: vancomycin, R: resistant, S: susceptible.

Well diffusion and microdilution methods in 96 microwell plates was used to assess the antimicrobial activity of essential oils. Briefly, Mueller Hinton agar plates were prepared with 6 mm diameter wells and inoculated with 106 colony-forming units (CFU/mL) of bacteria. A volume of 50 µL of each essential oil was added to each well, and well containing distilled water was used as a control. After a 24-hours incubation period at 37°C, the inhibitory diameters around the wells were measured.

Fisher Scientific (UK) sterile 96-well microtiter plates were used to measure the minimum inhibitory concentrations (MIC). A volume of 100 µL of the tested bacteria is incubated with 100 µL of tested essential oil at dilution ranging from 0.25 to 64 % (v/v), added to each well of a 96-well microplate. Plates were incubated at 37 °C for 24 hours. The MIC was the lowest dilution of essential oil that inhibits bacterial growth.

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JCHR (2025) 15(1), 189-195 | ISSN:2251-6727

On the other hand, wells without bacterial growth were inoculated onto essential oil-free agar plates to determine the minimum bactericidal concentrations (MBC). After 24 hours of incubation at 37°C, the MBC is defined as the lowest dilution of essential oil with no visible bacterial growth. The MBC/MIC ratio was calculated to assess whether the essential oil is bactericidal or bacteriostatic.

4. Results

Chemical composition

The chromatographic profiles of *T. vulgaris* essential oil are illustrated in Fig. 1 and in table 2. According to these results, *T. vulgaris* essential oil is mainly composed of thymol at 27.28%, followed by phenol, 2-methyl-5-(1-methylethyl)- at 21.32%, and benzene, 1-methyl-2-(1-methylethyl)- at 20.2%.

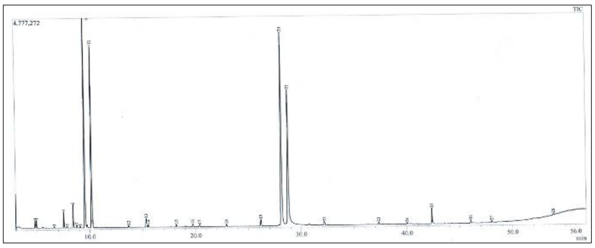

Fig. 1. Chromatographic profiles of *Thymus vulgaris* essential oil

Table 2. Chemical composition of *T. vulgaris* essential oil

Peak	Name	Retention time	Area %
1	Biclyclo[3.1.0]hex-2-ene,2-methyl-5-(I-methylethyl)-	4.808	0.60
2	IR-alpha.Pinene	4.934	0.60
3	Biclyclo[3.1.1]heptane,6,6-dimethyl-2-methylene-,(IS)-	6.619	0.10
4	Beta.-Myrcene	7.521	1.39
5	Alpha-Phellandrene	7.830	0.20
6	1,3-Cyclohexadiene,I-methyl-4-(Imethylethyl)	8.404	2.09
7	Limonene	8.760	0.28
8	Beta Phyllandrene	9.064	0.14
9	Benzene,I-methyl2-(I-methylethyl)-	9.538	20.32
10	3-Octanone	9.726	0.30
11	1,4-Cyclohexadiene,I-methyl-4-(methylethyl)-	10.178	18.75
12	Biclyclo[3.1.0]hexan-2-ol,methyl-5-(I-methylethyl)-,(I.alpha.,2.beta.,5.alpha)-	13.651	0.21
13	1,6-Octadien-3-ol,3,7-dimethyl	15.307	0.89
14	Biclyclo[3.1.0]hexan-2-ol,methyl-5-(I6methylethyl)-,(I.alpha.,2 alpha..,5.alpha)-	15.517	0.31
15	3-Cyclohexen,Iol, 4--methyl-I(I-methylethyl)-	18.143	0.31
16	3-Cyclohexene,I-methanol I-,.alpha., alpha.4-trimethyl-	19.725	0.30
17	Benzene,I-methoxy-4-methyl-2-(I-methylethyl)-	20.384	0.24
18	2;5-Cyclohexadiene-1,4-dione,2-methyl-5-(I-methylethyl)	22.907	0.27
19	Caryophyllene	26.173	0.86
20	Thymol	28.134	27.28
21	Phenol,2-methyl-5-(I-methylethyl)	28.763	21.81
22	Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methlene-,[S-(R*,S*)]-	32.182	0.47
23	Cycloheptane,4-methlene-I-methyl-I-propen-I-yl)-I-vinyl	37.333	0.25
24	Diethyl phthalate	40.008	0.14
25	Phenol,4-methoxy-2,3,6-trimethyl-	42.350	1.31
26	1,2-Benzedicarboxylic acid,bis(2-methylpropyl)ester	46.350	0.31
27	Dibutyl phthalate	48.017	0.14
28	Hexatriacontane	53.878	0.16

Antimicrobial effects

The antibacterial effects of the essential oil against S. aureus strains are represented in Fig. 2. and Table 3. The essential oil has an excellent antibacterial effect and they appear to be efficient in killing bacteria at low doses based on the MBC values and the MBC/MIC ratio. The inhibitory diameter ranging from 48.67±1.53 to 52.5±1.32 mm, MIC values from 1 to 2 µL/mL, and MBC values ranging from 1 to 4 µL/mL. Since the MBC/MIC ratio is always inferiors of 4, the essential oil has a bactericidal effect rather than bacteriostatic.

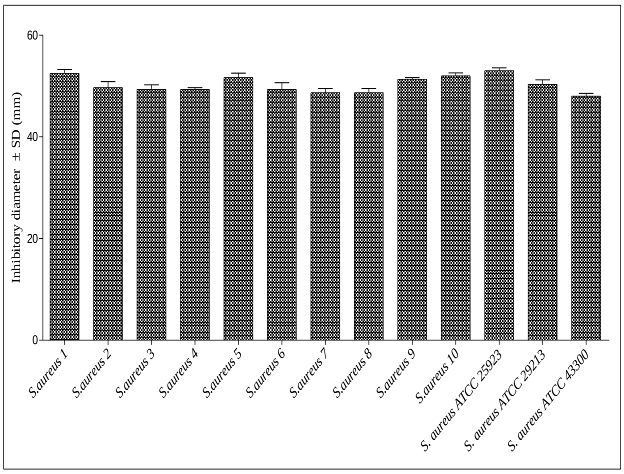

Fig. 2. Inhibitory diameters of *T. vulgaris* essential oil towards standard and clinical S. aureus strains

Table. 3 MIC (µL/mL), MBC (µL/mL), and MBC/MIC ratio of *T. vulgaris* essential oils

Bacterial strains	MIC	MBC	MBC/MIC
S. aureus 1	1	2	2
S. aureus 2	1	1	1
S. aureus 3	1	2	2
S. aureus 4	1	2	2
S. aureus 5	2	4	2
S. aureus 6	1	2	2
S. aureus 7	1	2	2

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JCHR (2025) 15(1), 189-195 | ISSN:2251-6727

S. aureus 8	1	2	2
S. aureus 9	1	2	2
S. aureus 10	1	1	1
S. aureus ATCC 25923	1	2	2
S. aureus ATCC 29213	1	2	2
S. aureus ATCC 43300	2	4	2

5. Discussion

Multidrug-resistant bacteria, especially *S. aureus*, are becoming more and more prevalent, which poses a serious threat to modern healthcare. Its capacity to develop resistance to multiple antibiotics has significantly hampered treatment efficacy, leading to prolonged illness, higher healthcare costs, and increased mortality rates [7, 25]. Alternative treatment strategies must be investigated and developed using natural products, particularly essential oils. Their complex chemical composition could target bacterial cells in a variety of ways, which lowers the chance that resistance may arise.

Based on the results of GC/MS, the chemical composition of *T. vulgaris* essential oil is mainly composed of phenol and thymol, along with 26 other compounds in minor concentrations. [26] has also found that thymol (15.52%) and Thymol methyl ether (10.78%) are the major compounds in the Romanian T. vulgaris essential oil. The significant variations observed in the chemical composition of the *T. vulgaris* essential oils can be related to the variations in the plant species, geographic locations, and environmental factors.

The essential oil exhibits a significant antibacterial effect towards all the tested strains of *S.aureus*. Different compounds may have different modes of action, in addition to the synergistic effects between the major and minor compounds of the essential oils. It has been found that the various compound of essential oils may affect the bacterial growth by damaging the cell walls and interfering with metabolic processes. The effectiveness of essential oil against S. aureus bacteria is probably due to the absence of lipo-polysaccharide layer.

Thymol, present in high concentrations in *T. vulgaris*, has been found to inhibit the bacterial growth by disrupting bacterial cell membranes, leading to cell lysis and death. It is particularly effective against Gram-positive bacteria such as *S. aureus* and *Bacillus subtilis* [27]. Phenol could also denatures bacterial proteins and enzymes, disrupting their cellular division [28].. Furthermore, some other minor components could interfere with quorum sensing systems, which is a regulatory mechanism used by bacteria to coordinate gene expression based on population density and environmental change. Essential oil compounds may disrupt the quorum sensing systems in various ways, including inhibiting signal molecule production, disrupting signal transduction, and binding to signalling receptors. These potentially mitigate bacterial pathogenicity by inhibiting virulence factor expression and biofilm formation [29].

In Conclusion, the chemical composition and the antibacterial properties of *T. vulgaris* essential oil indicate that its primary constituents are thymol (21.32%), phenol, 2-methyl-5-(1-methylethyl), and benzene, 1-methyl-2-(1-methylethyl). These bioactive components possess strong antimicrobial activities, possibly accounting for the considerable antibacterial activity. The essential oil shown significant effectiveness against both standard and multidrug-resistant strains of *S.aureus*. The findings highlight the efficacy of *T. vulgaris* essential oil as a natural antibacterial agent, especially against drug-resistant bacterial infections. Future research should investigate the synergistic effects of its components and evaluate their usefulness in clinical and pharmacological contexts.

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