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Isolation, characterization and antimicrobial activity study of *Thymus vulgaris*

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Herbal medicines are important for ensuring sustainable development goals (SDGs) in healthcare, particularly in developing countries with high rates of antimicrobial resistance (AMR) and little access to medical facilities. *Thymus vulgaris* is a widely used herbal medicinal plant known for its secondary metabolites and antimicrobial properties. The present study involved a comprehensive examination of the isolation, characterization, and antibacterial activity of *Thymus vulgaris* obtained from Ethiopia. The aerial part of the plant *Thymus vulgaris* was successively extracted with hexane, chloroform, and methanol based on differences in polarity. Phytochemical screening tests conducted against hexane, chloroform and MeOH crude extracts indicated the presence of some secondary metabolites. Based on the thin-layer chromatography tests, the chloroform extract was subjected to column chromatography, yielding Tv-2 compounds, namely 5-isopropyl-2-methylphenol. The structures of the compounds were elucidated via spectroscopic methods (UV–Vis, FT-IR and NMR). We investigated the antibacterial properties of hexane crude extract, chloroform crude extract, MeOH crude extract, and isolated fractions derived from *T. vulgaris* against various bacterial strains. This study contributes to a better understanding of the bioactive components present in *Thymus vulgaris* crude extracts and their potential role in tackling microbial infections.

Keywords *Thymus vulgaris*, Phytochemical analysis, Secondary metabolites, Antimicrobial activity

Antimicrobial resistance (AMR) is a global challenge that affects humans, animals, and plants without geographical boundaries or species barriers[1]. To support the United Nations' Sustainable Development Goals (SDGs), which include eliminating poverty, promoting well-being, ensuring food security, reducing discrimination, improving employment opportunities, and promoting global prosperity, preventing AMR is key[2]. AMRs pose a serious threat to the SDGs in low-income countries by compromising social well-being and healthcare systems. Intentional engagement is necessary to address the rising impact of antimicrobial resistance on sustainable development goals, as demonstrated by rising healthcare costs and economic development[3,4].

Antimicrobial resistance is associated with the Sustainable Development Goals recognized by the United Nations (UN), namely, manipulating health (SDG-3) as well as various societal, environmental, and economic goals, highlighting the importance of addressing AMR. The growing problem of antibiotic resistance requires a comprehensive, interdisciplinary strategy known as **"one health"**. Resistant pathogens and antimicrobial agents are widespread, affect humans, animals, plants, and the environment and have the potential for species-to-species and border-to-border transmission[5,6].

In alignment with the Sustainable Development Goals of the UN, the One Health concept underlines the interdependence of animal, environmental, and human health and represents a comprehensive social approach. The prominent purpose of one's health perception is to assure multidisciplinary groups of multiple fields, including agriculture, environment, and health, with the purpose of increasing medical products for humans, animals and plants[7].

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The ability to effectively treat a wide range of ailments caused by bacteria, parasites, viruses, and fungi is gradually endangered by antimicrobial resistance. AMR weakens the effectiveness of antibacterial, antiparasitic, antiviral, and antifungal medications, increasing the cost or adaptation of treatment for high-risk patients. Its effects are particularly evident among the most vulnerable populations, who have a decreased risk of persistent illness and increased mortality[8,9].

Traditional and conventional medicine are dependent on the custom of medicinal plants, a practice with a rich history. Any plant species that contains chemical components in one or more of its parts that can be utilized medicinally or as ingredients to make significant pharmaceuticals qualifies as a medicinal plant[10]. This definition allows for the variation between medicinal plants with scientifically established therapeutic properties and constituents and those that are considered medicinal but have not undertaken comprehensive scientific exploration. Recently, an increasing number of studies have confirmed the diverse chemical structures found in natural products, mostly emphasizing their antibacterial activities[11,12].

Several plants have been utilized in traditional medicine for a long time. The selection process considers the significance of public health, supporting evidence of effectiveness and safety, and comparative cost-effectiveness[13]. Medicinal plants satisfy the priority health care needs of a population. Medicinal plants can save lives, reduce suffering and improve health, but only when they are of good quality, safe, effective, available, and properly used by prescribers and patients. These medicines are proposed to be available within the context of functioning health systems at all times in adequate amounts, in appropriate dosage forms, with assured quality and adequate information and at low price for individuals and the community[14].

In Ethiopia, especially in Oromo culture, there is high concern about traditional medicinal plants. Oromo, a large population group that occupies a large area in Ethiopia, explains the importance of medicinal plants by their proverbs in their local language**, "Muka beekan qoricha hin se'an",** which is translated into several of the trees you are familiar with that possess medicinal qualities, so it is wise not to disregard them and venture too far[15].

A typical method for obtaining bioactive substances from therapeutic plants involves Soxhlet extraction[16,17]. It involves continuous extraction and evaporation cycles, allowing for effective extraction of bioactive molecules that are difficult to isolate. Soxhlet extraction is preferred over contemporary techniques due to its efficiency, cost-effectiveness, eco-friendliness, and ability to prevent thermal degradation[18,19].

The Lamiaceae/Labiatae family, which includes more than 236 genera and approximately 7200 species, is quite diverse. The Thymus genus stands out in the Lamiaceae family in particular because of its large number of species. The rich chemical structure of thyme herbs, which includes flavonoids, phenolic molecules, aromatic compounds, saponins, tannins, iridoids, and quinones, significantly contributes to its ethnomedicinal importance[20,21].

Thymus vulgaris (Fig. 1), an aromatic herb and member of the Lamiaceae family, has a long history of application in traditional and modern medicine, as well as in the pharmaceutical industry. Thyme is indigenous to Europe, particularly the Mediterranean region, and is widely planted throughout the world. This short, bushy herb features small, elliptical, greenish-gray leaves with short leaves. Thyme produces a fragrance distinct from that of thymol and is commonly used as a culinary herb. The active component is the essential oil, with thymol and carvacrol as the primary compounds[22].

Traditionally, various Thymus species have been utilized in Ethiopia. The fresh or dried leaves of these species are locally used for flavoring and are infused into herbal tea. They play a crucial role in the production of berbere and shirro, a mixture of pepper and bean/pea powder, as well as baaduu, a type of fermented cottage cheese. Within the realm of traditional medicine, Thymus species are utilized for the treatment of a variety of

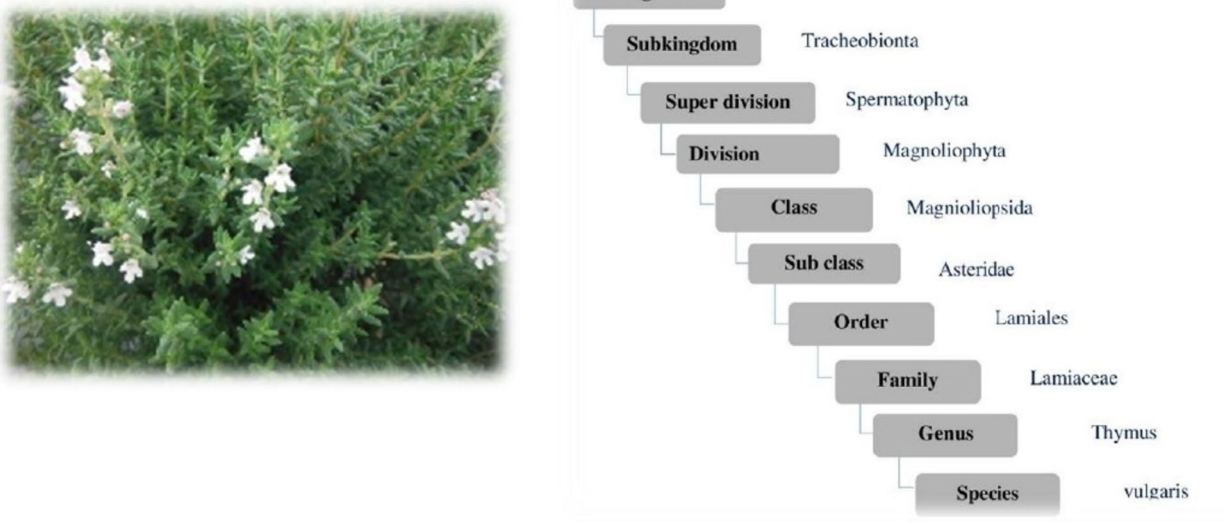

Fig. 1. Taxonomic position of *Thymus vulgaris* plants.

conditions, such as gonorrhea, respiratory issues, hepatic and renal disorders, stomach pain, hypertension, renal complications, and dermal fungal infections[23,24].

This research examined the chemical composition and medicinal application of *Thymus vulgaris* collected from Ethiopia, a developing country where antimicrobial resistance is challenging. To the best of our knowledge and based on a literature search, no research has been conducted on the isolation, characterization and antimicrobial activity of bioactive compounds from *Thymus vulgaris* collected by researchers from the study area. In this study, we highlight the value of investigating and using indigenous herbal medicines considering their traditional significance and their applications in modern pharmacology through isolation, characterization and antimicrobial activity studies of *Thymus vulgaris*.

Materials and methods

Fresh *Thymus vulgaris* plants were obtained from a local medicinal herb in May 2023 in the central city of Ethiopia, Addis Ababa. Sample preparation was performed at Dambi Dollo University, Ethiopia. The collected plants were rinsed with running tap water, air-dried, and subsequently pulverized into a fine powder using an electric blender (Bosch Limited, Germany). The resulting powder was then stored in a polyethylene bag for further analysis. Crude extraction, phytochemical screening, isolation and purification of compounds, characterization of pure compounds using spectroscopic techniques such as Ultraviolet–visible spectroscopy (UV–Vis), Fourier Transform Infrared Spectroscopy (FT-IR), and Nuclear Magnetic Resonance (NMR) were conducted at IIT Hyderabad, India, due to the facility of the necessary instrumentation. Antimicrobial activity assays were performed at the Central Drug Research Institute, India.

All the organic solvents and chemicals used for the phytochemistry tests were purchased from commercial sources at Sisco Research Laboratories, Ltd. Ltd., Avra, and Sigma Aldrich. All the organic solvents hexane, chloroform, MeOH, distilled water, and ethyl acetate were purified by the solvent distillation method. NMR spectra were collected using a Bruker AVANCE III spectrometer with TMS as a reference. ^{1}H NMR and ^{13}C NMR data were collected at 600 MHz. Resonances are reported in parts per million (ppm), and coupling constants, J, are reported in hertz (Hz). Infrared spectra were recorded by the KBr pellet method using JASCO FT-IR instruments. A Soxhlet apparatus, rotary evaporator, TLC plate, TLC chamber, column chromatography column of different sizes, Whatman N**o**. 1, flasks of different sizes, UV lamb and UV–Vis spectra, bacterial strains for antimicrobial testing and appropriate media were used in this research work.

Sample preparation and crude extraction

First and foremost, it is imperative to initiate a stage of evaluation to assess available ethnomedicinal research, chemotaxonomic data related to a specific medicinal plant, information sourced from various historical records, and traditional knowledge obtained from local healers and experts[25,26]. The collected aerial parts of *Thymus vulgaris* were washed several times, dried in an open-air environment, and shielded from direct sunlight to prevent degradation of the bioactive herbal components. Once air-dried, the aerial parts were finely ground using an analytical mill to obtain a consistent particle size. Finally, the powdered aerial portions of *T. vulgaris* were packed into polyethylene bags for further processing. This process ensures that the plant part is clean, dry, and properly stored for further extraction.

Subsequently, 150 g of powdered *Thymus vulgaris* was extracted by hexane using a Soxhlet apparatus for 6 h at 45 °C. This involves extracting the material 1 gm of plant powder in to 5 ml of organic solvent ratios. After filtration, the hexane filtrate was concentrated using a rotary evaporator at 40 °C under reduced pressure. The resulting defatted residue was then air-dried at room temperature for further extraction. Next, 121.3 g of the defatted residue was collected after hexane crude extraction for a second extraction using chloroform using the same procedure above. Finally, 86.2 g of the dried residue from the chloroform extraction step was collected for a third extraction using methanol following the same procedure. All the crude extract was then stored at 4 °C until analysis.

Determination of the crude extract percentage yield

The crude extract percentage yield was determined using the following formula[27]:

$$\text{Percentage yield} = \frac{\textit{Weight of extract}}{\textit{Weight of the sample}} \text{X } 100\%$$

The percentage of the crude extract obtained relative to the initial amount of plant material used in the extraction process is given. This calculation helps in assessing the efficiency of the extraction process and provides valuable information for further experimentation or processing of the crude extract.

Phytochemical screening tests

Thymus vulgaris crude extract were subjected to phytochemical screening. Hexane crude extract, Chloroform crude extract, and MeOH crude extract were among the plant extracts that are initially screened for the study using the prescribed standard procedures[28,29].

Test for Terpenoids When 5 ml of the extracts were combined with 2.5 ml of $CHCl_3$ and 3 ml of concentrated H_2SO_4, a reddish-brown color formed at the interface, indicating a positive terpenoid test result.

Test for Saponins The presence of saponins was determined by diluting all extracts (0.5 g each) with distilled water to 20 ml and vigorously shaking them in a graduated cylinder for 15 min. Foam formation was observed as an indicator of the presence of saponins.

Test for Quinones The hexane, $CHCl_3$, and MeOH crude extracts (0.2 g each) underwent individual treatment with alcoholic potassium hydroxide solution, resulting in the formation of a blue color from red, indicating the presence of quinones.

Test for Steroids The crude extract was subjected to the Liebermann–Burchard test by heating to a boil, followed by cooling and mixing with a few drops of acetic anhydride. Upon injecting concentrated sulfuric acid from the sides of the test tube, a brown ring formed at the intersection of the two layers, with a positive test result for steroids indicated by an upper layer turning green.

Test for Phenols After treating the hexane, $CHCl_3$, and MeOH crude extracts (0.2 g) with 3–4 drops of ferric chloride solution, the presence of phenols was determined by determining the formation of a bluish black color.

Cardiac Glycoside Test In the Keller-Killiani test, the test solution was treated with a few drops of glacial acetic acid and ferric chloride solution. Following the addition of concentrated sulfuric acid, two layers developed: a reddish-brown bottom layer and an upper layer of acetic acid that turned bluish green, indicating the presence of glycoside.

Test for Flavonoids Five millilitres of diluted ammonia solution was added to an aliquot of the plant extract obtained by hexane, $CHCl_3$, and MeOH as an aqueous filtrate. Concentrated H_2SO_4 was then added. The appearance of a yellow color confirmed the presence of flavonoids, which disappeared upon standing.

Test for Tannins Approximately 0.25 g of the extract was boiled in 10 ml of water in a test tube and subsequently filtered. A blue–black or brownish-green color was observed when a few drops of 0.1% Fe_2O_3 solution were added, indicating the presence of tannins.

Test for alkaloids For the alkaloid test, approximately 0.25 g of the crude extracts of hexane, CHCl3, and MeOH were mixed in 5 ml of diluted hydrochloric acid. The mixture was then filtered. Wagner's reagent (iodine in potassium iodide) was applied to the filtrates in two millilitres. The formation of a brown or reddish precipitate indicates the presence of alkaloids.

Isolation and characterization of the bioactive molecule

In our experimental approach, we used Soxhlet crude extraction, followed by isolation and purification via column chromatography. The purified extract was subsequently characterized using spectroscopic techniques such as FT-IR, NMR, and UV–Vis to analyze its bioactive structure. Among the three crude extracts (hexane, chloroform, and MeOH), chloroform was selected due to its superior separation compared to the hexane and methanol extracts. The crude extracts were chromatographed using a glass column with an internal diameter of 1.0 inches and a height of 18 inches. In the column, approximately 12 g of the crude extract was subjected to chromatography with 40 g of medium-sized silica gel (200 mesh) serving as the stationary phase 13 fractions were obtained. The elution process involved sequentially increasing the polarity of the n-hexane and ethyl acetate mixtures as eluents (ranging from 3:1), aiming to identify the most suitable solvent for checking complete spot resolution.

The purity of each of the collected fractions was monitored using TLC. Among the 13 fractions obtained from the chloroform crude extract, the second (Tv-2) and seventh fractions (Tv-7) appeared pure based on TLC observations, while the others exhibited mixed spots. Subsequent NMR analysis revealed that the seventh fraction contained impurities and was consequently unsuitable for molecular characterization. Ultimately, the second fraction was confirmed to be pure after NMR analysis and was chosen for further spectral data collection. While our objective was to identify and characterize as many compounds as possible, it is important to recognize that certain compounds may have been ignored due to impurities or the complexity of plant chemistry.

Screening for antimicrobial activity

Screening for antimicrobial activity involves various methods to identify potential compounds for combating infectious diseases. Traditional techniques like well-diffusion and broth-dilution are commonly used but may have limitations in reproducibility and speed. The hexane crude extract, chloroform crude extract, methanol crude extract and some isolated fractions from *Thymus vulgaris* tests against five bacterial strains one gram-positive bacterium, *Staphylococcus aureus* ATCC 29,213 and four gram-negative bacteria *Escherichia coli* ATCC 25,922, *Acinetobacter baumannii* BAA 1605, *Pseudomonas aeruginosa* ATCC 27,853, and *Klebsiella pneumoniae* BAA 1705 using Muller-Hinton broth II agar dilution method.

Minimum inhibitory concentration (MIC) for ESKAPE pathogens

As per the European Committee for Antimicrobial Susceptibility Testing (EUCAST)/CLSI, the broth dilution method was used to determine the minimum inhibitory concentration (MIC) of compounds against rapidly growing bacterial pathogens. Cation supplemented Muller-Hinton broth II was used as a medium. In brief, 100 µL was added in well to serve as a sterility control well. Further, 50 µL were added to another well and served as a control well. Then, 50 µL of each dilution (2:1) of antibiotic, were added to the respective well and further inoculated with colonies of bacteria. The inoculation was performed in such a way so that it could contain 5×10^5 CFU/mL. The concentration at which there is no visible growth of bacteria was observed was taken as MIC.

The bacterial strains utilized in the experiment were obtained from the stock cultures and streaked onto Mueller Hinton plates, followed by an incubation period of at 35–37 °C for 18–24 h. After incubation well separated bacterial colonies were selected as inoculums. The transfer of bacteria was conducted using a bacteriological loop onto autoclaved Mueller Hinton agar that has been cooled to approximately 45 °C in water bath and mixed by gently swirling the flasks. Subsequently, the medium was then poured to sterile Petri dishes, allowed to solidify and used for the biotest[30]. All the samples prepared for bacteriological tests were dissolved in 0.1% DMSO in miliQ-water by bath sonicating for 1 h to make the stock solutions. The antimicrobial properties of the isolates were tested against five standard strains of bacterial pathogens.

Results and discussion

The air-dried powdered leaves of *Thymus vulgaris* (150 g) were pulverized and extracted with hexane at 45 °C for 6 h by a Soxhlet apparatus to yield a dark green extract (28.7 g, 19.2%). After hexane extraction, 121.3 g of defatted powder was weighed and extracted with chloroform at 45 °C for 6 h using the same apparatus with petroleum ether to yield a green extract (35.1 g, 28.9%). Finally, after chloroform extraction, 86.2 g of defatted powder was extracted with methanol at 45 °C with a Soxhlet apparatus for 6 h to yield a blue black extract (33.8 g, 39.2%). Based on their polarity, three organic solvents (hexane, chloroform, and methanol) were chosen; the average values of crude extracts percentage yield are calculated for each organic solvent.

Phytochemical screening results

Phytochemical screening tests conducted against all crude extracts indicated that hexane extracts constitute saponins, terpenoids, steroids, and cardiac glycosides. Chloroform extracts include saponins, terpenoids, phenols, and cardiac glycosides. Methanol extracts contain alkaloids, saponins, tannins, terpenoids, phenols, and cardiac glucosides. On the other hand, aqueous extracts contain saponins, alkaloids, terpenoids, phenols, and cardiac glycosides. The phytochemical screening test results for the hexane, $CHCl_3$ and MeOH crude extracts of *T. vulgaris* showed the following results (Table 1).

Characterization of Tv-2: Compound **Tv-2** was isolated from the $CHCl_3$ crude extract of *Thymus vulgaris and characterized*. The Tv-2 compound was isolated as a reddish pink solid with an Rf value of 0.23 in hexane: ethyl acetate (3:1). Structure elucidation of the compound was based on the spectroscopic data obtained from FT-IR, NMR (1H-NMR and 13C-NMR) and UV–Vis spectral data. TLC (n-hexane: ethyl acetate, 3:1 v/v): Rf (0.23; ^{1}H NMR (600 MHz, $CDCl_3$: δ 7.01 (d, J = 7.7 Hz, 1H) 6.70 (d, J = 8.9 Hz, 1H) 6.60 (s, 1H) 5.65 (s, 1H) 2.76 (m, J = 13.8, 6.8 Hz, 1H) 2.18 (s, 3H) 1.17 (d, J = 6.94 Hz, 6H); ^{13}C NMR (600 MHz, CDC_3): δ 153.50, 148.33, 130.90, 121.31, 118.81, 113.20, 33.65, 23.95, 15.44; IR (KBr pellet): 3392 cm^{-1}, 3018 cm^{-1}, 2958 cm^{-1}, 2885 cm^{-1}, 1621 cm^{-1}, 1582 cm^{-1}, 1511 cm^{-1}, 1503 cm^{-1}, 1454 cm^{-1}, 1421 cm^{-1}, 1112 cm^{-1}, 993 cm^{-1}, 864 cm^{-1}, 811 cm^{-1}, 755 cm^{-1} : UV–Vis: λ max 274 nm. Experimental data obtained from FT-IR and UV–Vis were converted into spectral graphs using OriginPro 9.0 64-Bit software, while NMR (^{1}H-NMR and ^{13}C-NMR) spectral data were plotted using MestReNova.

FT-IR spectrum of Tv-2

The IR spectrum of **Tv-2** is shown in Fig. 2. In the **Tv-2** spectrum, clear characteristic absorption bands were observed at 3392 cm^{-1}, which are characteristic of γOH groups associated with aromatic rings. The band at 3018 cm^{-1} was characteristic of the γC–H relationship characteristic of an aromatic ring. The band at 2958 cm^{-1} was characteristic of the γCH3 group, which was confirmed by an additional band at 2885 cm^{-1}. The absorption band at 1621 cm^{-1} was characteristic of an absorption band of a conjugate double γC=C– bond. The intense absorption band at 1582 cm^{-1} was characteristic of a carbon skeleton with an aromatic structure and was more intense at approximately 1500 cm^{-1} (in this case, 1503 cm^{-1}). The bands at 1582, 1511, 1503, 1454, and 1421 cm^{-1} were characteristic of aromatic rings, and the band at 1421 cm^{-1} was characteristic of γC–OH bonds in phenols. The bands at 1112, 993, and 864 cm^{-1} were characteristic of aromatic rings with substituents at positions 1, 2, and 5, respectively. The band at 811 cm^{-1} was typical for an *m*-substituted aromatic ring, and that at 755 cm^{-1} was typical for an *o*-substituted ring. From the conclusions drawn, it could be concluded that this compound belongs to the phenol group, which has a CH_3 group in the *o*-position and a $C(CH_3)_2$ group in the *m*-position, which was confirmed by the structural formula of **Tv-2.**

The ^{1}H-NMR (600 MHz, $CDCl_3$) spectrum (Fig. 3) of compound **Tv-2** showed the presence of aromatic, alkene, alcohol hydroxyl, and aliphatic protons. The aromatic part of this compound contains three protons that are chemically nonequivalent. These three signals appeared at δ 7.01 (1H, d (J = 7.7 Hz)), δ 6.70 (1H, d (J = 8.9 Hz)) and δ 6.61 (1H, s). The signal at δ 5.65 indicated the presence of a hydroxyl proton, which is directly attached to the benzene ring. Moreover, the spectrum of this compound shows δ 2.76 (1H, m (J = 13.8, 6.8 Hz)) and δ 2.18 (3H, s), which indicates the presence of two different methane and methyl protons, both of which are attached

Test	Crude extracts			
	Hexane	Chloroform	MeOH	Aqueous
Terpenoids	+	+	+	+
Saponins	+	+	+	+
Quinones	–	–	–	–
Steroids	+	–	–	–
Phenols	–	+	+	+
Cardiac Glycosides	+	+	+	+
Flavonoids	+	–	–	+
Tannins	–	–	+	+
Alkaloids	–	–	+	+

Table 1. Phytochemical screening results for *T. vulgaris.* +ve presence of phytochemical constituents –ve absence of phytochemical constituents.

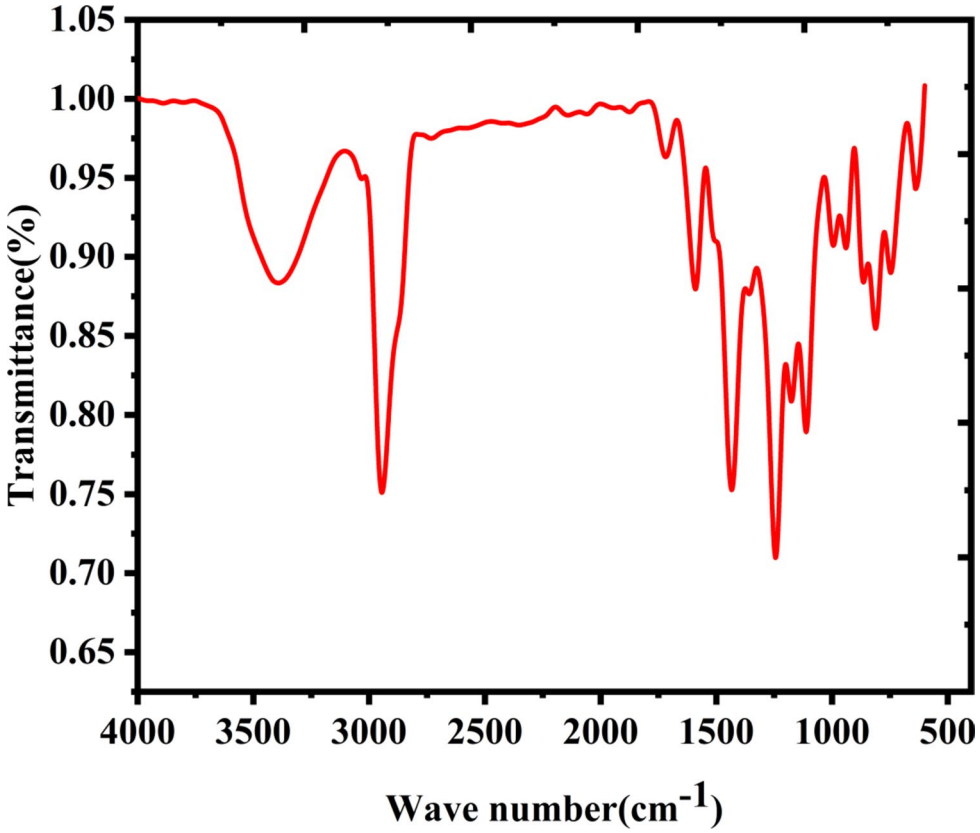

Fig. 2. FT-IR spectrum of **Tv-2**.

to the phenyl ring. In addition, the spectrum of this compound shows two 3H doublets at δ 1.17 (J = 6.94 Hz), which revealed the presence of two methyl protons.

Figure 4 shows the ^{13}C NMR spectrum of compound **Tv-2**, which shows well-resolved aromatic carbon, hydroxyl carbon and nonoxygenated aliphatic carbons. ^{13}C-NMR of the compound revealed that the **Tv-2** compound has ten carbon atoms. In the aromatic region, there are six nonequivalent carbon signals at δ 113.20, 118.81, 121.31, 130.90, 148.33, and 153.50. In the aliphatic region, there is one carbon signal at δ 33.65, which is a methine carbon directly attached to the phenyl ring. The peak at δ 23.95 represented two aliphatic carbons that are chemically equivalent. Additionally, the signal at δ 15.44 indicated the presence of a methyl carbon attached to the aromatic ring. Therefore, the ^{13}C NMR data showed the presence of aliphatic, alcoholic and aromatic carbons that exactly matched the previously identified FT-IR functional groups.

The UV–Vis spectrum of **Tv-2** (Fig. 5) shows an absorption band at 274 nm, indicating the presence of an aromatic ring attached by a hydroxyl functional group through a π–π* electronic transition.

Based on the above UV–Vis, FT-IR, and NMR spectroscopic data, the structure of **Tv-2** was determined to be **5-isopropyl-2-methylphenol** (Fig. 6).

After obtaining the ^{13}C NMR spectral data, we utilized the CSEARCH spectral similarity search platform to determine the structure of Tv-2. Among the one hundred thirty-six organic compounds generated from CSEARCH, carvacrol has been proposed to be one of the most promising compounds[31]. Previous research has extensively explored the chemical composition of this medicinal plant. Studies have consistently identified carvacrol as a major constituent of the essential oils extracted from various cultivars of *Thymus vulgaris*[32]. Furthermore, chemical analysis of *Thymus vulgaris* essential oil has consistently revealed the presence of carvacrol in combination with thymol[33]. Additionally, isolation techniques involving hydrodistillation, fractionation, and purification have been employed to extract carvacrol from *Thymus vulgaris,* along with other compounds such as thymol and linalool[34]. Carvacrol, also known as 5-isopropyl-2-methylphenol, is one of the components identified in *T. vulgaris* and is commonly referred to as Tv-2.

Antimicrobial assay

The hexane crude extract, chloroform crude extract, methanol crude extract and some isolated fractions from *Thymus vulgaris* showed the following results of inhibition against five bacterial strains one gram-positive bacterium, *Staphylococcus aureus* ATCC 29,213 and four gram-negative *bacteria Escherichia coli* ATCC 25,922, *Acinetobacter baumannii* BAA 1605, *Pseudomonas aeruginosa* ATCC 27,853, and *Klebsiella pneumoniae* BAA 1705 using the agar dilution method (Table 2).

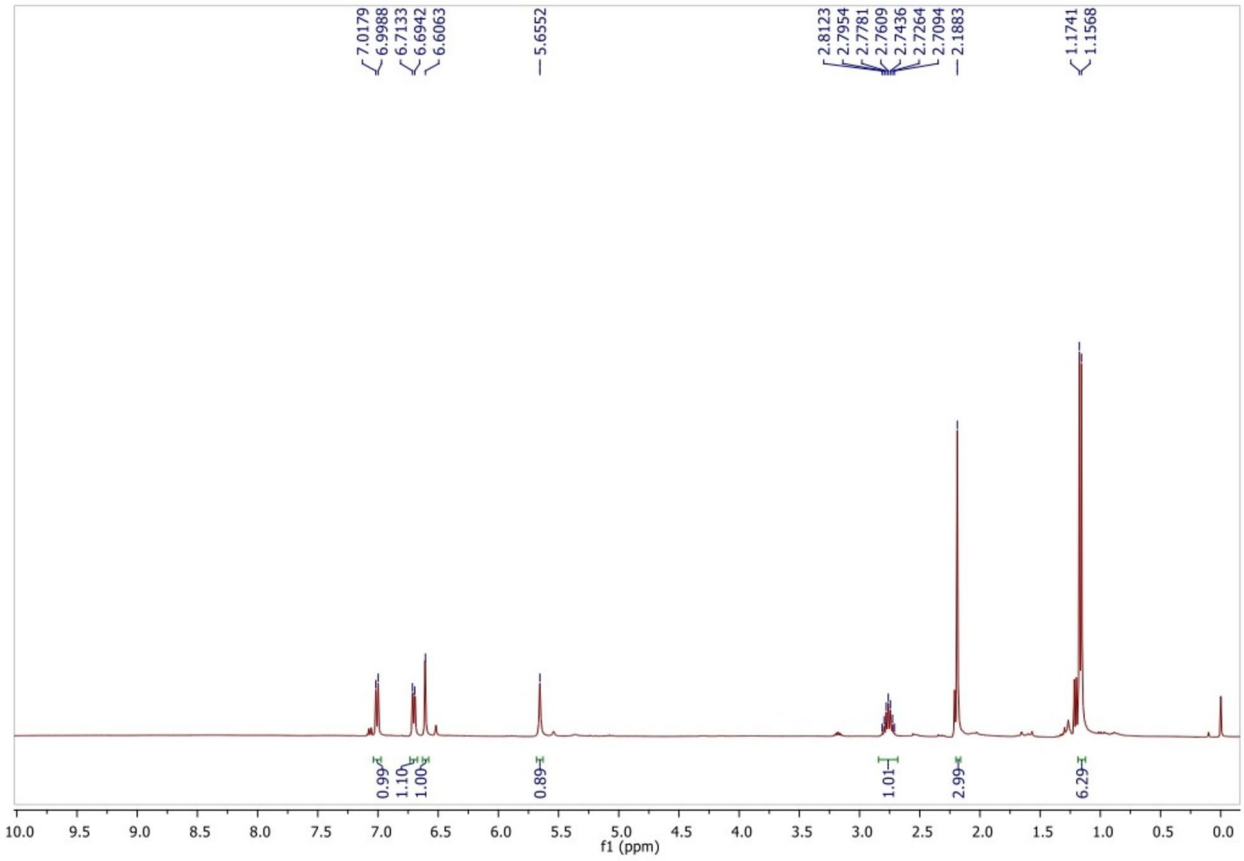

Fig. 3. ^{1}H-NMR spectral data of **Tv-2** in $CDCl_3$.

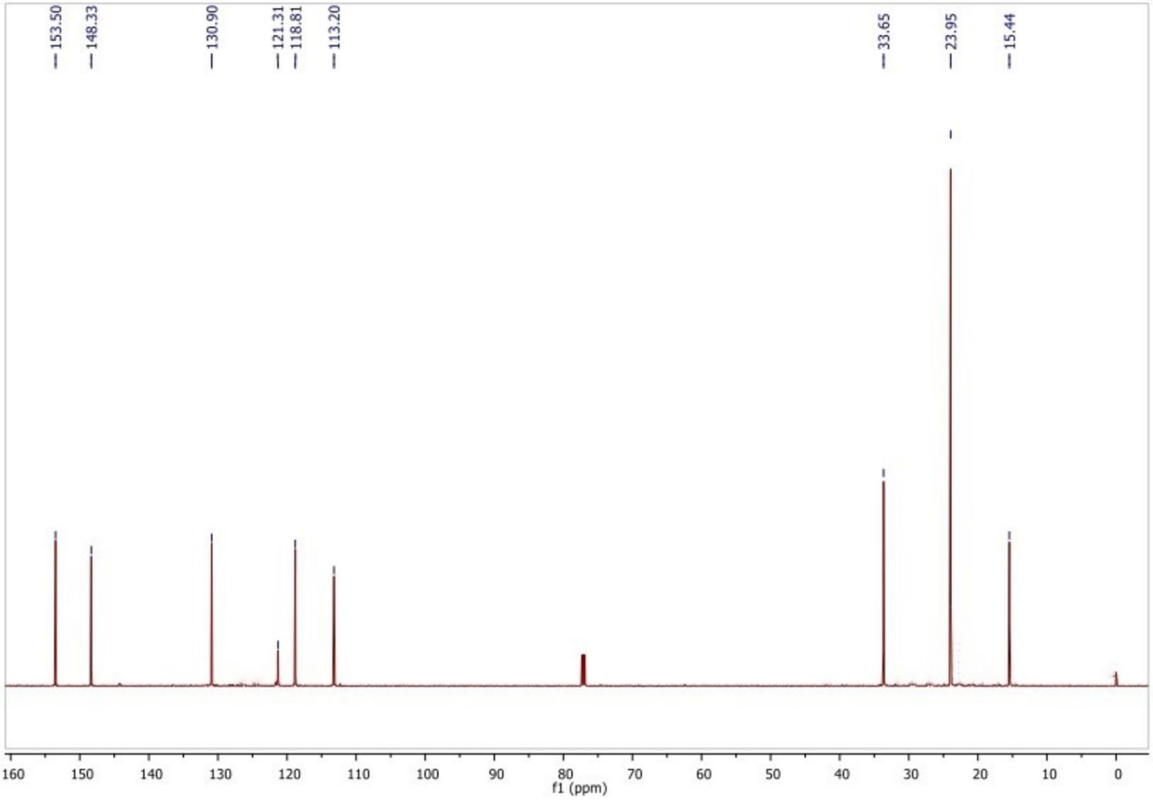

Fig. 4. ^{13}C-NMR spectral data of **Tv-2** in $CDCl_3$.

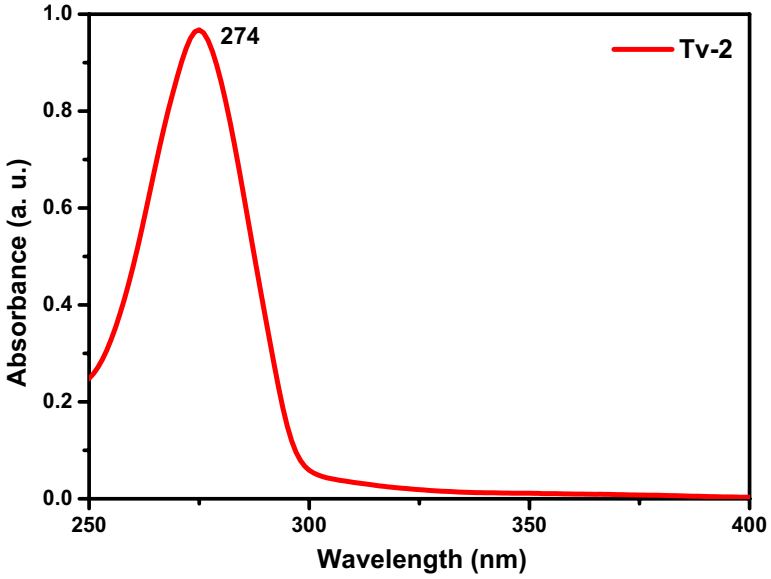

Fig. 5. UV–Vis spectral data of **Tv-2**.

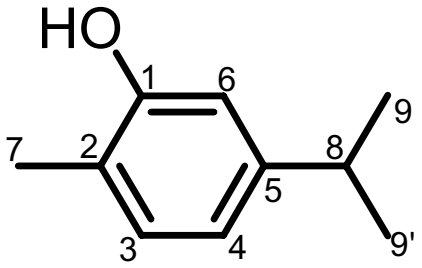

Fig. 6. The proposed structure of **Tv-2**.

MIC (µg/mL)							
Sample	**Amount**	**Solubility**	***E.coli* ATCC 25,922**	***S.aureus* ATCC 29,213**	***K. pneumoniae* BAA 1705**	***A. baumannii* BAA 1605**	***P.aeruginosa* ATCC 27,853**
A-1	2.5 mg	DMSO	> 64	> 64	> 64	> 64	> 64
A-2	2.5 mg	DMSO	> 64	> 64	> 64	> 64	> 64
A-3	2.5 mg	DMSO	> 64	> 64	> 64	> 64	> 64
Tv-1	2.5 mg	DMSO	> 64	> 64	> 64	> 64	> 64
Tv-2	2.5 mg	DMSO	> 64	> 64	> 64	> 64	> 64
Tv-3	2.5 mg	DMSO	> 64	> 64	> 64	> 64	> 64
Tv-4	2.5 mg	DMSO	> 64	> 64	> 64	> 64	> 64
Tv-5	2.5 mg	DMSO	> 64	> 64	> 64	> 64	> 64
Tv-6	2.5 mg	DMSO	> 64	> 64	> 64	> 64	> 64

Table 2. Minimal inhibitory concentrations (MICs) of some selected *T. vulgaris* bacteria. A-1, hexane extract; A-2, chloroform extract; A-3, methanol extract; Tv-1, fraction-1; Tv-2, fraction-2; Tv-3, fraction-3; Tv-4, fraction-4; Tv-5, fraction-5; Tv-6, fraction-6; MIC, minimum inhibitory concentration, DMSO, dimethyl sulfoxide.

Extracts of *Thymus vulgaris* showed significant antibacterial activity against pathogenic bacteria. The MICs of T. vulgaris hexane crude extract, chloroform crude extract, methanol crude extract, fraction-1, and fraction-2, which are Tv-2, fraction-3, fraction-4, fraction-5, and fraction-6, for all the bacterial strains were greater than 64 µg/mL. Sharif and Shallal separately conducted research demonstrating that *Thymus vulgaris* oil exhibits potent antimicrobial activity against *Streptococcus sanguinis*, *Porphyromonas gingivalis*, and *Prevotella intermedia*[35,36],

Cutibacterium acnes, *Staphylococcus epidermidis*[37]. Researches showed that *Thymus vulgaris* bioactive compounds are effective against various microbes such as *Escherichia coli*, *Salmonella typhimurium*, *Enterococcus faecalis*, *Staphylococcus aureus*, and methicillin-resistant *Staphylococcus aureus*[38], *Candida albicans, and candida famata,* commonly found in urinary tract infections in dogs and cats[39], *mammaliicoccus lentus* and *salmonella thyphi*[40]. Based on Galgano et al., in vitro test *Thymus vulgaris* essential oil exhibited complete inhibition growth of *Escherichia coli* and *Staphylococcus aureus*[41]. According to Antih et al. and his coworkers, Thyme essential oil also shows antibacterial activity against respiratory pathogens such as *Haemophilus influenzae*, *Staphylococcus aureus*, and *Streptococcus pyogenes* in both the liquid and vapor phases[42]. However, our results were not in accordance with those of other reports. This discrepancy may be due to differences in the samples used, the extraction process, the composition of the samples used and the solubility process (water or solvents). Researchers suggest that the antimicrobial activity of bioactive components from *Thymus vulgaris* relies on both the quantity of bioactive components and the specific types of microorganisms involved[43].

Conclusion and future perspectives

The extraction of natural products using Soxhlet apparatuses has attracted increasing interest because of its categorization as a conventional method during the past decade, with increasing attention given to environmental, economic and safety considerations. The three organic solvents hexane, chloroform, and methanol were used to successively extract the thyme herb using a Soxhlet apparatus based on their polarity differences. The chloroform crude extract was subjected to column chromatography using increasing polarities of hexane and ethyl acetate mixtures, which resulted in the isolation of thirteen fractions, including the characterized compound **5-isopropyl-2-methylphenol,** from fraction 2 by using different spectroscopic techniques. These findings indicate the potential of *Thymus vulgaris* crude extract as a natural antibacterial agent with broad-spectrum activity against various pathogenic bacteria. We hope that other researchers will be inspired to collect all the information required in this field by what is missing in our research paper to explore additional applications by synthesizing nanoparticles for conducting biosensors and evaluating the cytotoxicity and antioxidant activities of *T. vulgaris*.

Data availability

The data are included in the article/supplementary material/referenced in the article.

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Author contributions

S.F. contributed to writing—original draft and conceptualization. R. M and S. C. perform the antimicrobial activity experiments. A.K, L.T. and K.R. contributed to writing—review and editing, data curation, and conceptualization. All authors reviewed the manuscript.

Competing interests

The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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