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Metabolite Profiling Using Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS) Techniques of Ethanolic Whole Plant Extract of *Thymus vulgaris* and Evaluation of Its Antibacterial Activity

A Scientific research

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

(وَقُل رَّبِّ زِدْنِي عِلْمًا)

صدق الله العلي العظيم

سورة طه : الآية (114)

DEDICATION

For my mother,

and

Brothers,

Thank you for your love and encouragement

For my father,

Thank you for your inspiration and never ending support

Acknowledgements

I would like to thank the many people in my life that helped me achieve this goal. First, I wish to acknowledge my supervisor, Abeer Fauzi Murad Al-Rubaye, for support and guidance leading to the completion of this dissertation. Second, I would like to express my deep appreciation to head of department and Dean of College of Science for Women for their support and encouragement.

ABSTRACT

Background: Medicinal plants have played an essential role in the treatment of various diseases. *Thymus vulgaris*, a medicinal plant, has been extensively used for biological and pharmaceutical potential. Metabolites are small molecules participating in metabolic reactions, which are necessary for cellular function, maintenance and growth. Typically, metabolites range from 50 to 1500 Da, while their concentrations span several orders of magnitude. The metabolome is highly dynamic, time-dependent, and metabolites are sensitive to many environmental conditions.

Objectives: The aims of our study: Phytochemical screening and identify some volatile compounds found in the Ethanolic whole plant extract of *Thymus vulgaris* via Using Fourier Transform Infrared Spectroscopy (FTIR). Using Gas Chromatography-Mass Spectrometry (GC-MS) Technique for Metabolite Profiling. Evaluate of Its Antibacterial Activity.

Materials and Methods: Dried plant parts were purchased from the local markets of Babylon Province, *Thymus vulgaris*, which were investigated in the advanced Botanical laboratory at the College of Science, the University of Babylon after cleaning and foreign substances isolation. An electrical grinder crushed them, and then the powder was collected in nylon bags and kept in the laboratory at room temperature until use. Separation and identification were performed on a GC-MS. In order to experimentally prepare for FTIR analysis, a suitable small amount of laboratory crushed leaf samples was converted into pellets using KBr. Here all experimental samples were subjected to three separate tests, with KBr pellets that were not actually treated serving as a control.

RESULTS : As part of the preparation for FTIR analysis, Peak (Wave number cm-^{l}), (Type of Intensity, Bond and Functional group assignment)

were 663.5 (Strong, C-Cl, alkyl halides), 687.5 (Strong, C-Cl, alkyl halides), 870.7 (Strong, =C–H, Alkenes), 920.9 (Strong, =C–H, Alkenes), 1017.01 (Strong, =C–H, Alkenes), 1047.0 (Strong, C-F, alkyl halides), 1095.15 (Strong, C-F, alkyl halides), 1244.17 (Stretch, C-F, alkyl halides), 1317.00 (Stretch, C-F, alkyl halides), 1373.19 (Strong, C-F, alkyl halides), 1608.00 (Strong, C-F, alkyl halides), 2335.50 (Strong, N-H, Amide), 1244.17 (C=C , Alkene), 1361.12 (Unknown), 2358.09 (Unknown). The identification of bioactive chemical compounds is based on the peak area, retention time molecular weight and molecular formula. GCMS analysis of *Thymus vulgaris* revealed the existence of the Cyclohexylaminopropylamino]ethylthi ophosphate, Formyl-L-lysine, Spiro[2.4]heptan-4-one, 4,4-Diphenyl-butyl)-(3phenylpiperidin-4, D-Glucose,6-O-αDgalactopyranosyl, Lucenin, 2,5-Dimethyl-4-hydroxy-3(2H)- furanone, 2,3-Diphenylcyclopro pyl)methyl pheny, Dodecanoic acid , 3-hydroxy, 2,6,10,14,18,22-Tetracosa hexaene, 10-Methyl-E-11-tridecen-1-ol, Isochiapin B, 1,3-Dioxolane , 4-[[(2-methoxy-4-octadecenyl)oxy]methyl]-2,2, E-9-Methyl-8-tridecen-2-ol ,acetate, E-9-Methyl-8-tridecen-2-ol ,acetate, 5-Hydroxymethylfurfural, 4-Hexenal , 6-hydroxy-4-methyl- , dimethyl acetal , acetate, α-D-Glucopyranoside , O-α-Dglucopyranosyl, 2-Cyclohexylpiperidine, and 3-O-Methyl-d-glucose.

It was investigated whether the secondary metabolites produced by *Thymus vulgaris* have antibacterial properties against three pathogens *Escherichia coli, Staphylococcus aureus* and *Proteus mirabilis*. In the current research, the biological activity of the ethanolic extract of whole plant *Thymus vulgaris*, as well as the conventional antibiotics Rifambin and Cefotoxime, against three different pathogens were investigated. *Escherichia coli* (10.04±0.14, 21.77±0.25, and 18.00±0.21), *Staphylococcus aureus* (08.71±0.12, 19.02±0.21, and 19.00±0.21), and

Proteus mirabilis (12.31±0.16, 22.04±0.23, and 23.85±0.27). *Thymus vulgaris* bioactive secondary metabolites were shown to show remarkable activity against *Proteus mirabilis*, with a mean value of (12.31±0.16).

Keywords: *Thymus vulgaris*, **FTIR, GCMS, Functional Groups, Secondary metabolites, Antibacterial Activity.**

INTRODUCTION

Plants have been used by man throughout history in a variety of ways. In scientific developments, there have been numerous studies on medicinal plants worldwide due to their therapeutic efficacy. Medicinal plants are an important source of lead compounds for developing new drugs that are highly effective, have no side effects, and are economically feasible [1, 2]. According to studies conducted by the World Health Organization, over 80% of the world's population relies on medicinal herbs for the treatment of various ailments. Plants have been used by humans for over 60,000 years based on fossil fuel records. Many studies have reported that anti-inflammatory, antibacterial, antifungal, antimalarial, anticancer, antioxidant, and other biological activities depend on a variety of phytoconstituents that are isolated from various medicinal plants [3]. Several exogenous factors, including drugs, chemicals, smoke, and environmental stress conditions, can produce reactive oxygen species (ROS). It has become clear that these factors are an important cause of diseases such as atherosclerosis, inflammation, neurodegenerative diseases, cardiovascular diseases, and cancer [4]. The excess production of free radicals such as OH+, O2−, etc. in the human body results in the damaging of cells. It causes severe oxidative damage to biological vital molecules such as lipid, deoxyribonucleic acid (DNA), and protein. Phenolic compounds are natural antioxidants that protect against free radical-induced illnesses. A variety of natural antioxidants contain phenolic compounds, such as curcuminoids, phenolics, lignans, tannins, coumarins, and flavonoids. As a matter of fact, antioxidants of natural origin have received considerable attention from the health and food industries in regard to identifying secondary metabolites. Antioxidants protect the body from radical damage by scavenging reactive oxygen species (ROS) [5]. Inflammation is a biological process

that is caused due to tissue injury, chemical irritation, and any sort of viral or pathogenic infection. This response is usually triggered by innate immune system receptors because of pain, redness, warmth, and swelling caused by pathogens.

Thymus vulgaris belongs to the Lamiaceae family and is widely used in folk medicines. It is mostly found in the Southern Europe Mediterranean, North Africa, and Asia regions with approximately 300 species [6]. Thymus vulgaris is the source of many phytoconstituents (secondary metabolites), such as phenols, tannins, glycosides, and flavonoids, which are responsible for many biological activities. The current study determines the phytochemical constituents through GC-MS and HPLC analyses of two fractions: ethyl acetate and n-butanol from T. vulgaris methanolic extract. The extracted plant was analyzed for various in vitro and in vivo biological assays, i.e., anti-inflammatory, antioxidant, hemolytic, thrombolytic, pyretic, and antidiabetic activities, along with a computational analysis on gastric cancer-causing genes by quantified phytochemicals.

The aims of our study:

- **Phytochemical screening and identify some volatile compounds found in the Ethanolic whole plant extract of *Thymus vulgaris* via Using Fourier Transform Infrared Spectroscopy (FTIR)**
- **Using Gas Chromatography-Mass Spectrometry (GC-MS) Technique for Metabolite Profiling.**
- **Evaluate of Its Antibacterial Activity.**

Literature Review

Secondary metabolites

Plants can use simple, inorganic precursors to synthesize a large diversity of low organic compounds. This synthetic capacity helps plants to colonize diverse and challenging environments. Low organic compounds are commonly separated by perspective function into primary metabolites, secondary metabolites (also called specialized metabolites or natural products), and plant hormones [7-10]. Primary metabolites are highly conserved and directly required for the growth and development of plants. Secondary metabolites, including major groups such as phenolics, terpenes, and nitrogen-containing compounds, are often lineage specific and aid plants to interact with the biotic and abiotic environment. Finally, plant hormones are defined as small compounds that regulate organismal processes, including the production of the other metabolites, by interacting with receptor proteins. Despite the fact that definitions of secondary metabolites are inherently diffuse, the distinction between primary metabolites, secondary metabolites, and plant hormones has found its way into textbooks and shapes our thinking in plant biology to this day [11]. An illustrative example is the field of plant–herbivore interactions, where major efforts have gone into disentangling how plants protect their primary metabolites (serving as nutrients for herbivores) using secondary metabolites (serving as defenses for plants), and how adapted herbivores manage to extract primary metabolites while avoiding the negative effects of secondary metabolites.

The biochemical coevolutionary arms–race theory, a key concept in plant–herbivore interactions, postulates that plant secondary metabolites evolve in response to herbivore pressure, resulting in the evolution of resistance mechanisms in herbivores. The resulting arms race is thought to drive the diversity of plant secondary metabolites and insect

herbivores [12]. Over the last decades, the distinction between primary metabolites, secondary metabolites, and plant hormones has proven a useful approximation. However, the emergence of a more detailed understanding of plant metabolism may require us to revisit this functional partitioning. In particular, an increasing number of genetic and functional studies on plant secondary metabolites are blurring the functional trichotomy by showing that plant secondary metabolites can have regulatory functions and serve as precursors for primary metabolites. In this review, we discuss this evidence, mostly focusing on examples that rely on the use of natural knockout variants, mutants, and transgenic plants altered in their capacity to produce certain secondary metabolites in combination with chemical complementation assays to demonstrate activity of the metabolites [13, 14]. We illustrate that for an increasing number of plant secondary metabolites, a strict functional separation from regulators and primary metabolites may not do them justice and possibly hinders our progress in understanding their roles for plant survival in hostile environments.

Secondary Metabolites as Regulators of Plant Defense Following early preliminary evidence of secondary metabolites regulating defenses, genetic evidence followed in 2009, when it was reported that Arabidopsis (Arabidopsis thaliana) mutants defective in indole glucosinolate biosynthesis no longer mount a callose defense response following Flg22 treatment. Callose formation is rescued by adding 4-methoxy-indol-3-ylmethylglucosinolate. The myrosinase PEN2 is required for this phenomenon, implicating glucosinolate breakdown in callose regulation. Shortly thereafter, it was discovered that indole-derived benzoxazinoid secondary metabolites have a comparable callose regulatory function in cereals. Benzoxazinoid-deficient bx1 maize (Zea mays) mutants are

defective in aphid- and chitosan-induced callose deposition, and callose induction is rescued by the addition of DIMBOA or DIMBOA-Glc. In both cases, the capacity to regulate callose is structurally specific and depends on the modification of the indole-derived ring. In Arabidopsis, indol-3-ylmethylglucosinolate, which lacks a methylated hydroxy-group on the aromatic ring, is inactive, whereas the methylated form is active. In maize, DIMBOA-Glc, which lacks a methylated hydroxy-group at the nitrogen, is active, whereas the methylated form (HDMBOA-Glc) is inactive. Whereas the callose response to benzoxazinoids is conserved between wheat (Triticum aestivum) and maize, they do not elicit callose in Arabidopsis, and intact glucosinolates do not elicit callose in maize. These studies show that callose regulation by secondary metabolites is highly specific, tightly controlled, and likely evolved repeatedly. The mechanism underlying secondary metabolite–induced callose formation awaits to be elucidated. Glucosinolates and benzoxazinoids may, for instance [15, 16], promote callose production by regulating hormonal pathways, through transcriptional regulation, or by directly initiating callose formation posttranslationally. Interestingly, glucosinolates and benzoxazinoids also seem to regulate the accumulation of other secondary metabolites. In Arabidopsis, mutants that are defective in the atypical myrosinase PEN2 release lower amounts of Trp-derived metabolites such as camalexin upon flg22 treatment and infection by Pseudomonas syringae. Furthermore, mutants defective in the CYP83B1 enzyme required for indole glucosinolate production also show lower accumulation of the phenylpropanoid sinapoylmalate. The phenylpropanoid phenotype is rescued in mutants that no longer produce the substrate of CYP83B1, indole-3-acetaldoxime, suggesting that it may be the aldoxime overaccumulation rather than the lack of downstream glucosinolates that suppresses sinapoylmalate. Suppressor screens

showed that the phenylpropanoid phenotype is also absent in plants that have mutated MEDa/b genes, which encode key components of a large multisubunit transcriptional complex that regulates phenylpropanoid biosynthetic genes. A recent study demonstrates that a group of Kelch Domain F-Box (KFB) genes that are involved in PAL inactivation are upregulated in indole glucosinolate mutants in a MED5- dependent manner, whereas PAL-activity is suppressed. PAL-activity and sinapoylmalate accumulation are (partially) rescued in glucosinolatedeficient KBF mutants. The model emerging from these studies is that aldoximes, which accumulate in CYP83B1 mutants, increase KFB-mediated PAL degradation through MED5 transcriptional regulation as well as other, yet unknown, mechanisms [17]. As aldoximes are produced by many different species, this form of defense regulation may also occur beyond glucosinolate-producing plants. Interestingly, wheat lines overexpressing a maize benzoxazinoid O-methyl transferase and thus accumulate more HDMBOA-Glc and less DIMBOA-Glc also show higher levels of the phenylpropanoid ferulic acid, despite unaltered pool sizes of amino acid precursors, suggesting that phenolic compounds may also be regulated by other secondary metabolite pathways.

Secondary Metabolites as Primary Metabolites

If secondary metabolites can regulate growth, development, and defense, can they also function as primary metabolites? Whereas primary metabolites are highly conserved, secondary metabolites evolve dynamically and are inherently variable in structure and production. This rapid evolution would seem to complicate their integration into the most fundamental workings of plant metabolism because it would require a rapid evolution of enzymes to connect these new structures into the more conserved metabolic pathways [18-23]. However, evidence for secondary

metabolites that are not strictly essential, but nevertheless contribute to primary metabolism, is emerging. In Arabidopsis, plants.

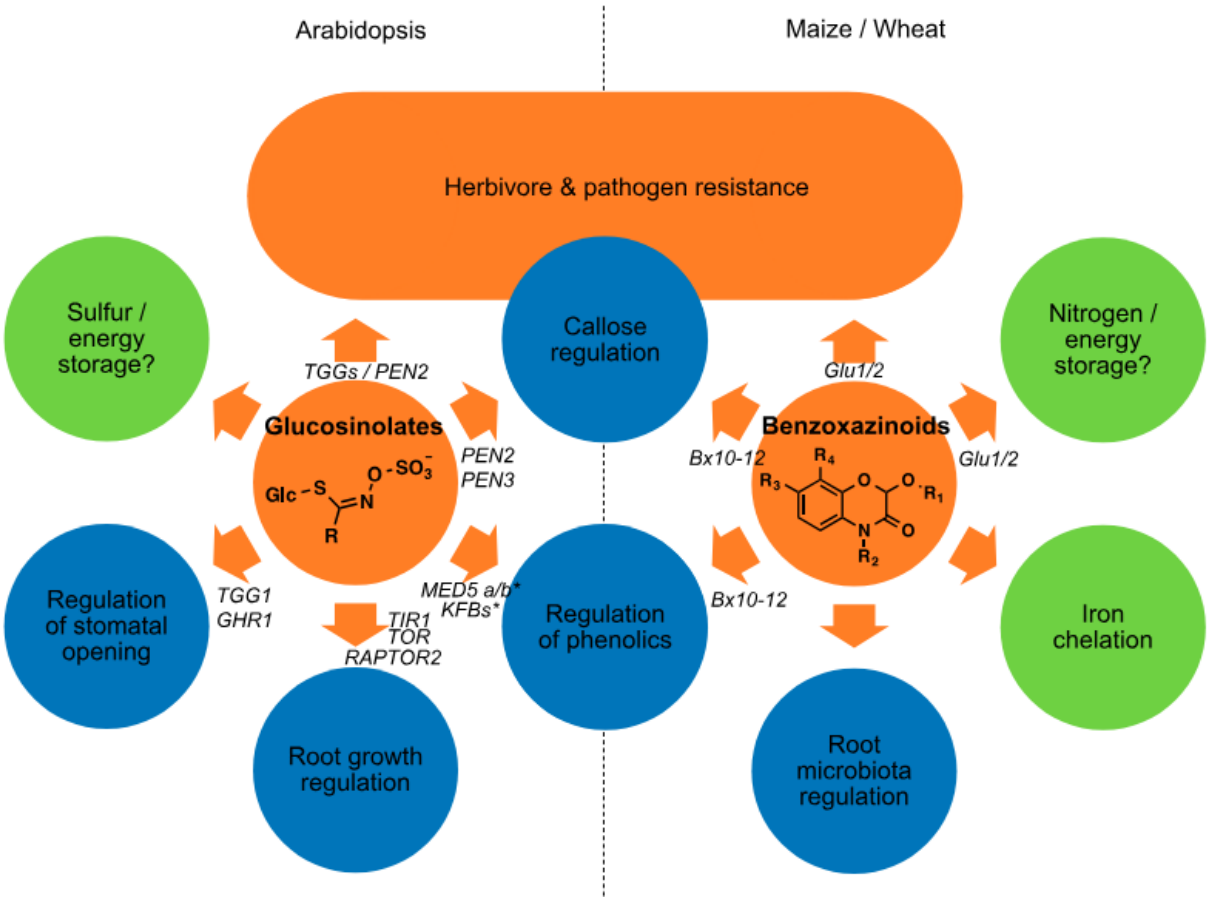


Figure 1. Glucosinolates and benzoxazinoids as examples of secondary metabolites that blurr the functional trichotomy of plant metabolism. Different functions of glucosinolates in Arabidopsis and benzoxazinoids in maize and wheat are depicted. Genes that are known to be involved in the different functions are indicated. Note that a direct role of benzoxazinoids and glucosinolates as plant primary metabolites (for instance, in the context of nitrogen/sulfur and/or energy storage) has not been clearly demonstrated so far. *MEDs and KFBs are likely regulated by aldoxime precursors of glucosinolates.

Metabolites

Metabolites are the intermediates and products of metabolism. The term metabolite is usually restricted to small molecules. Metabolites have various functions, including fuel, structure, signaling, stimulatory and inhibitory effects on enzymes, catalytic activity of their own (usually as a cofactor to an enzyme) [24, 25], defense, and interactions with other organisms. Plant produce a vast and diverse assortment of organic compounds the great majority of which do not appear to participate directly in growth and development.

These substances, traditionally referred to as secondary metabolites, often are differentially distributed among limited taxonomic groups within the plant kingdom. The evolving commercial importance of secondary metabolites has in recent years resulted in a great interest particularly in the possibility of altering the production of bioactive plant metabolites by means of tissue culture technology [26-30].

Plant cell and tissue culture technologies can be established routinely under sterile conditions from explants, such as leaves, stems, roots, and meristems [31] for both the ways for multiplication and extraction of secondary metabolites. In-Vitro production of secondary

Metabolite in plant cell suspension cultures has been reported from commercial medicinal plants. Plant secondary metabolites are unique sources for pharmaceuticals, food additives, flavors, and other industrial materials1 and the useof plant cell cultures has overcome several inconveniences for the production of these secondary metabolites. Organized cultures [32, 33], and especially root cultures, can make a significant contribution in the production of secondary metabolites. Secondary metabolites are described as an organic compounds which were not directly involved in the normal growth, development, or reproduction of an organism [34]. These metabolites are those

metabolites which are often produced in a phase of subsequent to growth, have no function in growth (although they may have survival function), are produced by certain restricted taxonomic groups of microorganisms, have unusual chemicals structures, and are often formed as mixtures of closely related members of a chemicalfamily.

Unlike primary metabolites, absence of secondary metabolites does not result in immediate death, but rather in long-term impairment of the organism's survivability, fecundity, or aesthetics, or perhaps in no significant change at all [35, 36]. These are often restricted to a narrow set of species within a phylogenetic group3. And these are also play an important role in plant defense against herbivory4 and other interspecies defenses5. Humans use secondary metabolites as medicines, flavorings, and recreational drugs in the recent past.

CLASSIFICATION

Secondary metabolites can be classified on the basis of chemical structure (for example, having rings, containing a sugar), composition (containing nitrogen or not), their solubility in various solvents, or the pathway by which they are synthesized (e.g., phenylpropanoid, which produces tannins). And usually classified according to their biosynthetic pathways [37, 38]. Three large molecule families are generally considered: Phenolics, Terpenes and Steroids, and Alkaloids, Flavanoids10.Some of them can have severe consequences.

Alkaloids are originally defined as pharmacologically active, nitrogen-containing basic compounds of plant origin. And they can block ion channels, inhibit enzymes, or interfere with neurotransmission, producing hallucinations, loss of coordination, convulsions, vomiting, and death.

Terpenes

Terpenes are among the most widespread chemically diverse groups of natural products. Terpenes are a unique group of hydrocarbon-based natural products whose structures may be derived from isoprene. Terpenes are classified by the number of 5-carbon units [39-41]. The function of terpenes in plants is generally considered to be both ecological and physiological: Allelophathy, Insecticidal, Insect pollinators, Plant hormone (Abscisic acid, gibberlellin).

Flavonoids

With more than 4500 different representatives known thus far, the flavanoids constitute an enormous class of phenolic natural products. Present in most plant tissues, often in vacuoles, flavonoids can occur as monomers, dimmers and higher oligomers [42]. Flavonoids comprise a diverse set of compounds and perform a wide range of functions. Specific flavonoids can also function to protect plants against UV-B irradiation. The flavonoids consist of various groups of plant metabolites which include chalcones, aurones, flavanones, isoflavonoids, flavones, flavonols, leucoanthocyanidins, catechins, and anthocyanins.

PRODUCTION OF SECONDARY METABOLITES AND RECENT IMPROVEMENTS OF PRODUCTION PROCESS

Plant secondary metabolites are unique resources for pharmaceuticals, food additives, and fine chemicals. They also provide original materials used in other areas. Besides direct extraction from plants, and chemical synthesis to provide those compounds or derivatives with similar uses [43], plant cell culture has been developed as a promising alternative for producing metabolites that are difficult to be obtained by chemical synthesis or plant extraction. However, in spite of

decades of efforts, production of plant secondary metabolites by plant cell culture technology is still facing many biological and biotechnological limitations.

One of the major obstacles is the low yield of plant secondary metabolites in plant cell cultures. Since the major roles of plant secondary metabolites are to protect plants from attack by insect, herbivores and pathogens, or to survive other biotic and a biotic stresses, some strategies for culture production of the metabolites based on this principle have been developed to improve the yield of such plant secondary metabolites and they include treatment with various elicitors, signal compounds, and a biotic stresses. Many such treatments indeed effectively promote the production of a wide range of plant secondary metabolites, both in vivo and in-Vitro. However, the productivity is still rarely competitive for industrial application.

MATERIALS and Methods

Plant Collection and Preparation

Dried plant parts were purchased from the local markets of Babylon Province, *Thymus vulgaris*, which were investigated in the advanced Botanical laboratory at the College of Science, the University of Babylon after cleaning and foreign substances isolation. An electrical grinder crushed them, and then the powder was collected in nylon bags and kept in the laboratory at room temperature until use. The whole plant of *Thymus vulgaris* was washed with fresh water and dried at room temperature for three days. After drying, 500 g of the whole plant was ground to a fine powder. T. vulgaris powder was packed in sealed plastic bottles until they were extracted [44].

Extraction Method

Dried whole plant powder of *Thymus vulgaris* (500 g) was macerated with methanol (1500 mL) for seven days using a Soxhlet extractor to obtain the methanolic extract. In the process of extracting and evaporating, rotary evaporators were used, and solid masses were formed that were amorphous [45, 46].

Gas Chromatography-Mass Spectrometry (GC-MS)

A gas chromatography-mass spectrometry (GC-MS) analysis of the methanolic extract of the *Thymus vulgaris* was carried out according to the protocols. A DB-5MS column with 0.25-μm film thickness, 0.25 mm in diameter, and 30 m in length was used to observe the methanolic extract using GC-MS model 7890B, 5977A working at 75 eV of the ionization energy. Helium was used as a carrier gas, which was used at a rate of 1 ml/min. We set the MS transfer line temperature to 280 °C, the split ratio to 1:6, injected 1 μL of the sample, and used a 30-atomic mass

unit mass scan. The columns were initially heated to 50 °C for one minute. A temperature rise of 8 °C per minute was set to reach 290 °C with regular intervals of time. With a 1-mL/min flow rate, helium was used as a carrier gas to move 1 mL of sample extract down the column. FID spectroscopy was used to identify and further analyze the components after separation in the column at 75 eV. To identify the molecular weight, name, and chemical structure of these compounds, we used NIST MS 2.0 libraries.

Fourier transform infrared spectroscopy (FTIR) analysis of *Thymus vulgaris*

A number of data already derived from an FTIR instrument were experimentally run and laboratory processed using mainly PC-based software in order to capture these FTIR spectra for each of the GLVs. In order to experimentally prepare for FTIR analysis, a suitable small amount of laboratory crushed leaf samples was converted into pellets using KBr, and at the same time a suitable and thin layer was created by physically pressing the studied mixture. In order to collect valid and studied information about the transmission of infrared light, at the same time data was actually collected in the wave number range including 4000 cm−1 to 500 cm^{-1}. Here all experimental samples were subjected to three separate tests, with KBr pellets that were not actually treated serving as a control.

Evaluation of the antibacterial efficacy of secondary metabolite chemicals against three pathogenic bacteria.

A sterile cork borer was used to create wells in the agar with a diameter of five millimeters. Then, 25 microliters of sample solutions containing metabolites generated by *Thymus vulgaris*, as well as the conventional antibiotics Rifambin and Cefotoxime, against three different pathogens were investigated *Escherichia coli*, *Staphylococcus aureus*, and *Proteus mirabilis*.

Statistical Analysis

If the p-value was less than or equal to 0.05, we used Student's t-test to find out whether the parametric data was statistically significant.

RESULTS and DISCUSSION

The plant kingdom produces hundreds of thousands of low molecular weight organic compounds. Based on the assumed functions of these compounds, the research community has classified them into three overarching groups: primary metabolites, which are directly required for plant growth; secondary (or specialized) metabolites, which mediate plant–environment interactions; and hormones, which regulate organismal processes and metabolism. For decades, this functional trichotomy of plant metabolism has shaped theory and experimentation in plant biology. However, exact biochemical boundaries between these different metabolite classes were never fully established. A new wave of genetic and chemical studies now further blurs these boundaries by demonstrating that secondary metabolites are multifunctional; they can function as potent regulators of plant growth and defense as well as primary metabolites sensu lato. Several adaptive scenarios may have favored this functional diversity for secondary metabolites, including signaling robustness and cost-effective storage and recycling. Secondary metabolite multifunctionality can provide new explanations for ontogenetic patterns of defense production and can refine our understanding of plant–herbivore interactions, in particular by accounting for the discovery that adapted herbivores misuse plant secondary metabolites for multiple purposes, some of which mirror their functions in plants.

As part of the preparation for FTIR analysis, Peak (Wave number cm^{-1}), (Type of Intensity, Bond and Functional group assignment) were 663.5 (Strong, C-Cl, alkyl halides), 687.5 (Strong, C-Cl, alkyl halides), 870.7 (Strong, =C–H, Alkenes), 920.9 (Strong, =C–H, Alkenes), 1017.01 (Strong, =C–H, Alkenes), 1047.0 (Strong, C-F, alkyl halides),

1095.15 (Strong, C-F, alkyl halides), 1244.17 (Stretch, C-F, alkyl halides), 1317.00 (Stretch, C-F, alkyl halides), 1373.19 (Strong, C-F, alkyl halides), 1608.00 (Strong, C-F, alkyl halides), 2335.50 (Strong, N-H, Amide), 1244.17 (C=C , Alkene), 1361.12 (Unknown), 2358.09 (Unknown).

The identification of bioactive chemical compounds is based on the peak area, retention time molecular weight and molecular formula. GCMS analysis of Cordia myxa revealed the existence of the Cyclohexylaminopropylamino]ethylthi ophosphate, Formyl-L-lysine, Spiro[2.4]heptan-4-one, 4,4-Diphenyl-butyl)-(3phenylpiperidin-4, D-Glucose,6-O-αDgalactopyranosyl, Lucenin, 2,5-Dimethyl-4-hydroxy-3(2H)- furanone, 2,3-Diphenylcyclopro pyl)methyl pheny, Dodecanoic acid , 3-hydroxy, 2,6,10,14,18,22-Tetracosa hexaene, 10-Methyl-E-11-tridecen-1-ol, Isochiapin B, 1,3-Dioxolane , 4-[[(2-methoxy-4-octadecenyl)oxy]methyl]-2,2, E-9-Methyl-8-tridecen-2-ol ,acetate, E-9-Methyl-8-tridecen-2-ol ,acetate, 5-Hydroxymethylfurfural, 4-Hexenal , 6-hydroxy-4-methyl- , dimethyl acetal , acetate, α-D-Glucopyranoside , O-α-Dglucopyranosyl, 2-Cyclohexylpiperidine, and 3-O-Methyl-d-glucose.

It was investigated whether the secondary metabolites produced by *Thymus vulgaris* have antibacterial properties against three pathogens. In the current research, the biological activity of the ethanolic extract of whole plant *Thymus vulgaris*, as well as the conventional antibiotics Rifambin and Cefotoxime, against three different pathogens were investigated. *Escherichia coli* (10.04±0.14, 21.77±0.25, and 18.00±0.21) Figure 2, *Staphylococcus aureus* (08.71±0.12, 19.02±0.21, and 19.00±0.21) Figure 3, and *Proteus mirabilis* (12.31±0.16, 22.04±0.23, and 23.85±0.27) Figure 4. *Thymus vulgaris* bioactive secondary

metabolites were shown to show remarkable activity against *Proteus mirabilis*, with a mean value of (12.31±0.16).

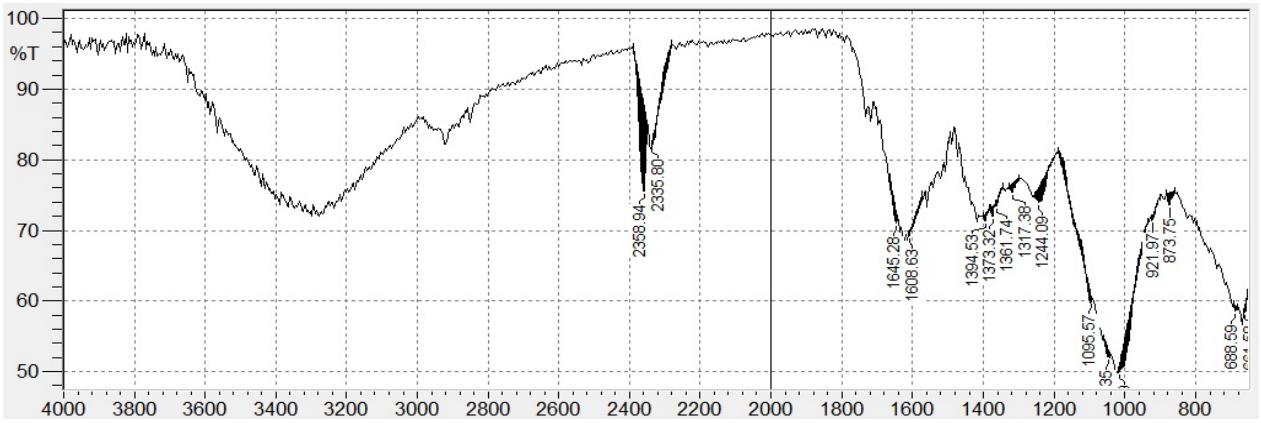
Secondary metabolites are the useful natural products that are synthesized through secondary metabolism in the plants. The production of some secondary metabolites is linked to the induction of morphological [47] differentiation and it appears that as the cells undergo morphological differentiation and maturation during plant growth. It is observed that in Vitro production of secondary metabolites is much higher from differented tissues when compared to non-differentiated or less –differentiated tissues.

There are lots of advantages of these metabolites like there is recovery of the products will be easy and plant cultures are particularly useful in case of plants which are difficult or expensive and selection of cell lines for high yield of secondary metabolites will be easy [48]. Many other examples could be presented with plant metabolic engineering as this research area is developing actively.

Metabolic engineering is probably a large step forward but playing on the genes will not solve all the problems that have prevented the development of commercial success in the field of plant secondary metabolites [49]. And Advances in plant cell cultures could provide new means for the cost-effective, commercial production of even rare or exotic plants, their cells, and the chemicals that they will produce. Knowledge of the biosynthetic pathways of desired compounds in plants as well as of cultures is often still rudimentary, and strategies are consequently needed to develop information based on a cellular and molecular level. Because of the complex and incompletely understood nature of plant cells in-Vitro cultures, case-by-case studies have been used to explain the problems occurring [50] in the production of

secondary metabolites from cultured plant cells. Advance research has succeeded in producing a wide range of valuable secondary phytochemical in unorganized callus or suspension cultures till to date; in other cases production requires more differentiated micro plant or organ cultures.

However, greater susceptibility of gram negatives against *Thymus vulgaris* oil than the gram positive bacteria is documented.9 The greater resistance of gram negatives might be associated with the presence of an outer membrane hydrophilic lipopolysaccharide, which inhibits accumulation of hydrophobic plant essential oil on the cell membrane [51]. Consumption of *Thymus vulgaris* flowers and leaves are safe; however, caution is warranted with the use of thyme oil, which should not be taken orally and should be diluted with a suitable oil (olive or almond oil) before use. Side effects of thyme oil if taken orally may include headache, dizziness, low blood pressure, gastrointestinal irritation, nausea, vomiting, and diarrhea.



Wavenumber (cm^{-1})

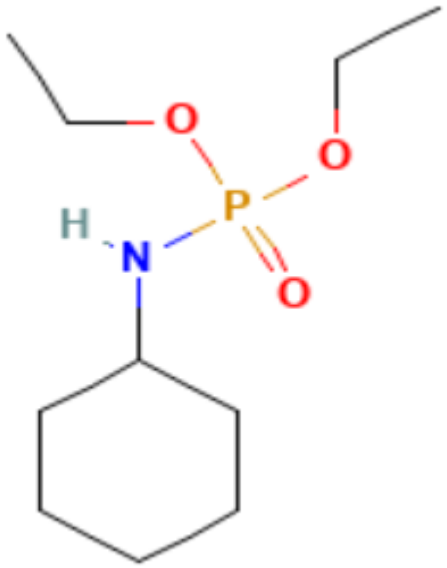
Figure 1. Fourier-transform infrared spectroscopic profile solid analysis of *Thymus vulgaris* .

Table 1. Fourier-transform infrared spectroscopic profile solid analysis of *Thymus vulgaris* .

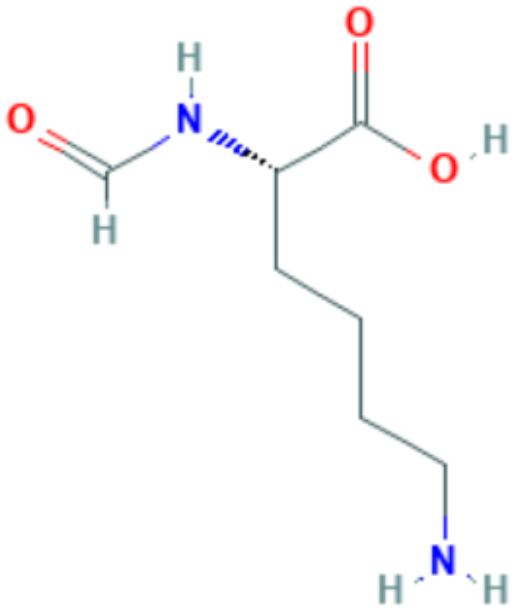
No.	Peak (Wave number cm-1)	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	663.5	58.395	0.968	663.51	653.87	2.174	0.054	Strong	C-Cl	Stretch	alkyl halides	600–800
2.	687.5	58.541	0.829	692.44	682.80	2.229	0.037	Strong	C-Cl	Stretch	alkyl halides	600–800
3.	870.7	73.620	1.751	883.40	860.25	2.950	0.114	Strong	=C–H	Bending	Alkenes	650-1000
4.	920.9	71.540	0.629	925.83	902.69	3.162	0.038	Strong	=C–H	Bending	Alkenes	650-1000
5.	1017.01	50.097	0.610	1018.41	937.40	18.291	0.617	Strong	C-F	Stretch	alkyl halides	1000-1400
6.	1047.0	52.070	1.052	1058.92	1041.56	4.834	0.102	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1095.15	60.041	0.613	1188.15	1093.64	14.288	0.082	Strong	C-F	Stretch	alkyl halides	1000-1400
8.	1244.17	73.963	2.094	1255.66	1190.08	7.368	0.356	Strong	C-F	Stretch	alkyl halides	1000-1400
9.	1317.00	75.349	1.346	1327.03	1300.02	3.175	0.093	Strong	C-F	Stretch	alkyl halides	1000-1400
10.	1361.12	73.525	0.515	1363.67	1344.38	2.382	0.009	Strong	C-F	Stretch	alkyl halides	1000-1400
11.	1373.19	72.091	1.372	1381.03	1365.60	2.110	0.047	Strong	C-F	Stretch	alkyl halides	1000-1400
12.	1394.01	71.347	0.989	1400.32	1382.96	2.465	0.049	Strong	C-F	Stretch	alkyl halides	1000-1400
13.	1608.00	69.356	0.346	1610.56	1571.99	5.532	0.107	Bending	N-H	Stretch	Amide	1550-1640
14.	1645.00	71.054	1.158	1664.57	1643.35	2.877	0.135	Variable	C=C	Stretch	Alkene	1620-1680
15.	2335.50	82.034	0.509	2337.72	2279.86	2.699	0.049	Unknown	-	-	-	-
16.	2358.09	75.576	11.981	2389.80	2349.30	3.022	1.175	Unknown	-	-	-	-

Table 2. GCMS analysis of ethanolic whole plant extract of *Thymus vulgaris*

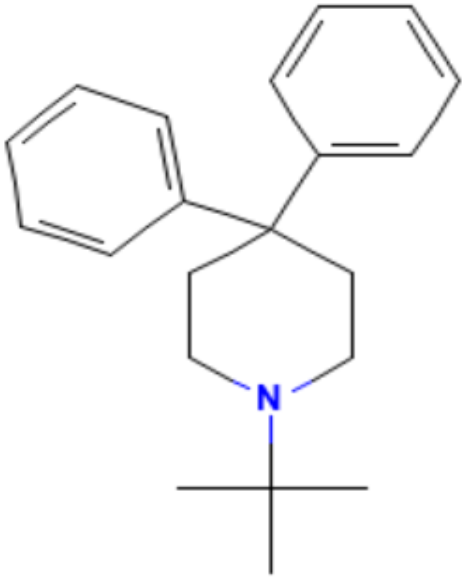
Compound	Molecular Formula	Molecular Weight
Diethyl cyclohexylphosphoramidate	$C_{10}H_{22}NO_3P$	235.26 g/mol
Formyl-L-lysine	$C_7H_{14}N_2O_3$	174.20 g/mol
1-tert-Butyl-4,4-diphenylpiperidine	$C_{21}H_{27}N$	293.4 g/mol
D-Glucose, 4-O-beta-D-galactopyranosyl	$C_{12}H_{22}O_{11}$	342.30 g/mol
Luteolin 6,8-di-C-glucoside	$C_{27}H_{30}O_{16}$	610.5 g/mol
2,5-Dimethyl-4-hydroxy-3(2H)- furanone	$C_6H_8O_3$	128.13 g/mol
phenylcyclopropyl)methanamine	$C_{10}H_{13}N$	147.22 g/mol
Dodecanoic acid, 3-hydroxypropyl ester	$C_{15}H_{30}O_3$	258.40 g/mol
6,9,12,15,18,21-Tetracosahexaenoic acid,	$C_{24}H_{36}O_2$	356.5 g/mol
12-Methyltridecanal	$C_{14}H_{28}O$	212.37 g/mol
Octanal, 7-methoxy-3,7-dimethyl	$C_{11}H_{22}O_2$	186.29 g/mol
Methyl 4,8,12-trimethyltridecanoate	$C_{17}H_{34}O_2$	270.5 g/mol
Ethyl 2-acetyldecanoate	$C_{14}H26O3$	242.35 g/mol
5-Hydroxymethylfurfural	$C_6H_6O_3$	126.11 g/mol
3-Methoxy-1-hydroxymethyl-adamantane	$C_{12}H_{20}O_2$	196.29 g/mol
4-O-alpha-D-Glucopyranosyl-moranoline	$C_{12}H_{23}NO_9$	325.31 g/mol
2-Cyclohexylpiperidine	$C_{11}H_{21}N$	167.29 g/mol
3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194.18 g/mol



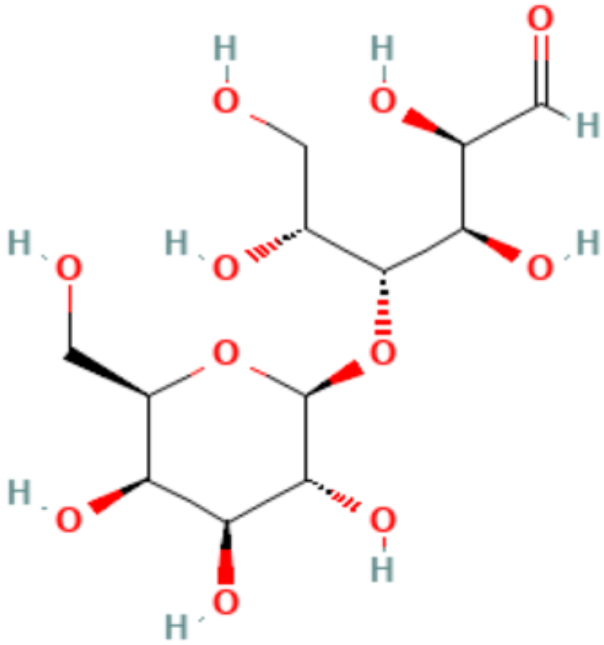
Diethyl cyclohexylphosphoramidate



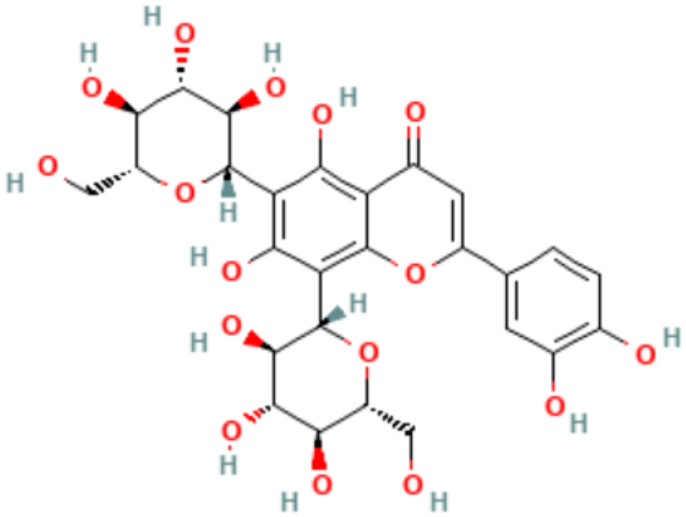
Formyl-L-lysine



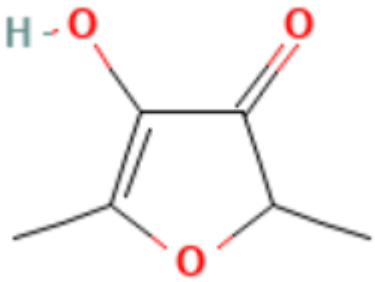
1-tert-Butyl-4,4-diphenylpiperidine



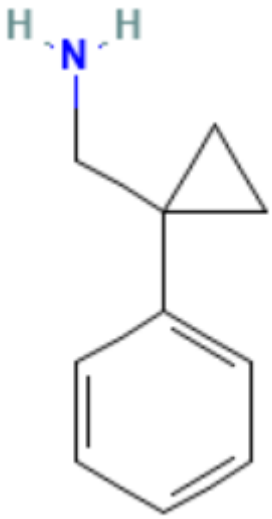
D-Glucose, 4-O-beta-D-galactopyranosyl



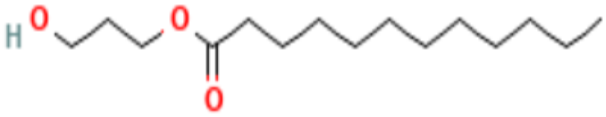
Luteolin 6,8-di-C-glucoside



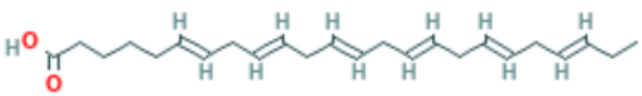
2,5-Dimethyl-4-hydroxy-3(2H)- furanone



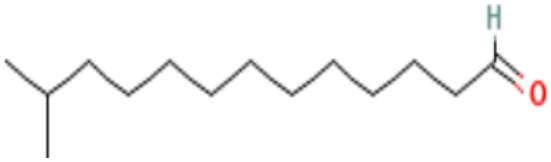
phenylcyclopropyl)methanamine



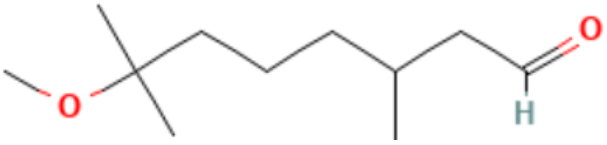
Dodecanoic acid, 3-hydroxypropyl ester



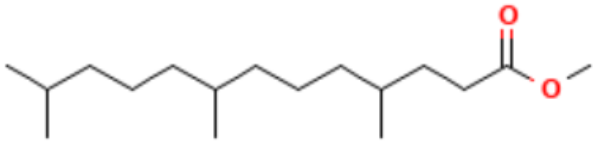
6,9,12,15,18,21-Tetracosahexaenoic acid



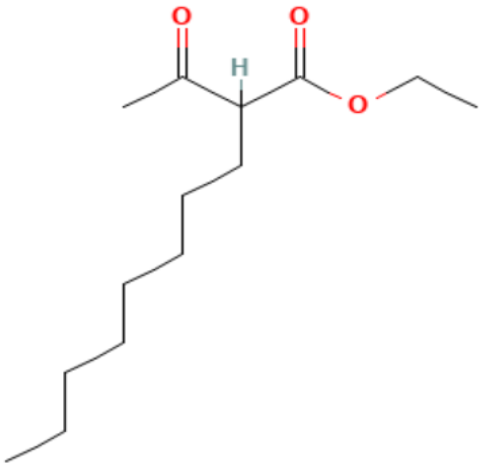
12-Methyltridecanal



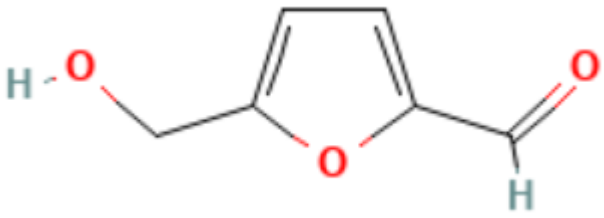
Octanal, 7-methoxy-3,7-dimethyl



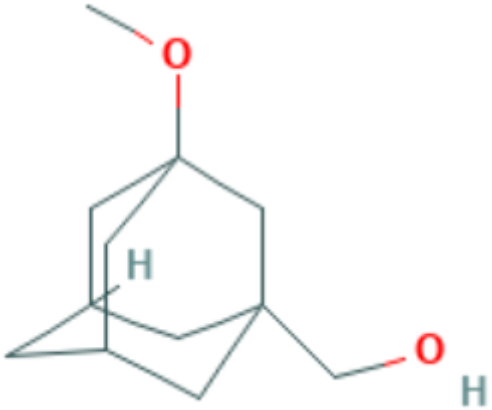
Methyl 4,8,12-trimethyltridecanoate



Ethyl 2-acetyldecanoate

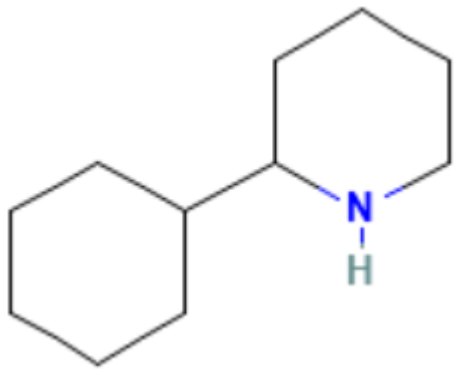


5-Hydroxymethylfurfural



3-Methoxy-1-hydroxymethyl-adamantane

4-O-alpha-D-Glucopyranosyl-moranoline



2-Cyclohexylpiperidine

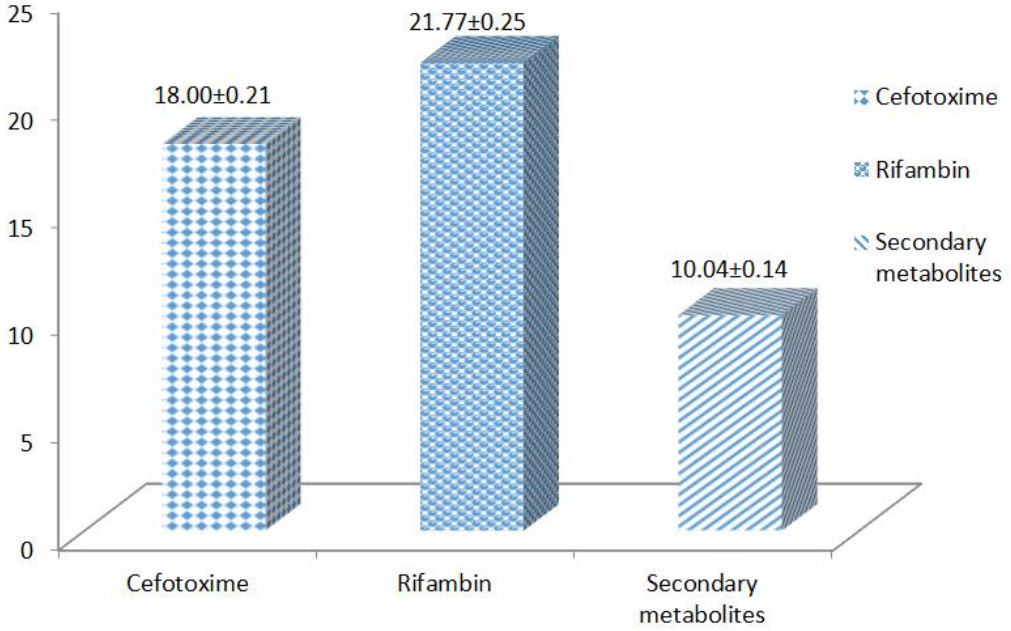


Figure 2. Bioactive metabolite products of *Thymus vulgaris* , Rifambin (Standard) and Cefotoxime (Standard) as anti-bacterial activity against *Escherichia coli.*

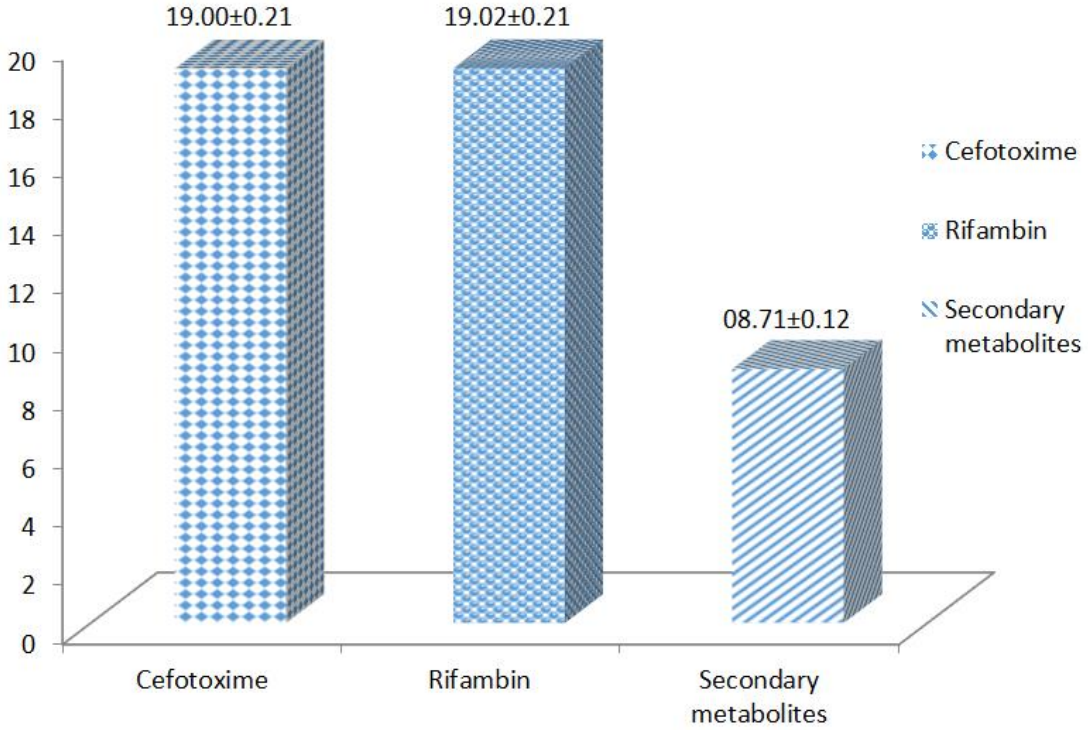


Figure 3. Bioactive metabolite products of *Thymus vulgaris* , Rifambin (Standard) and Cefotoxime (Standard) as anti-bacterial activity against *Staphylococcus aureus*.

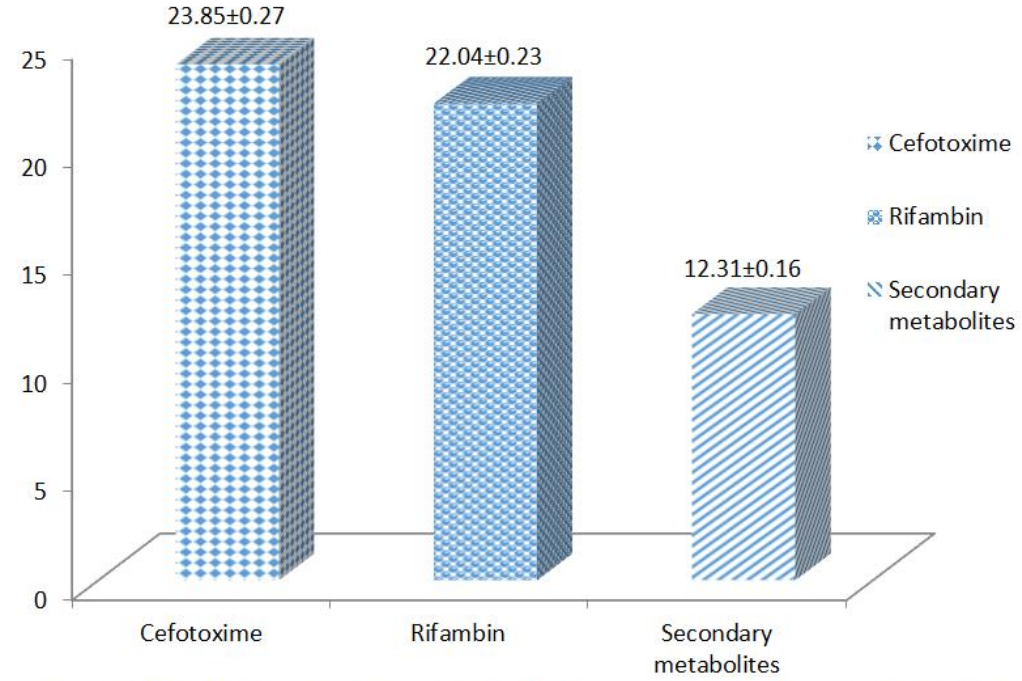


Figure 4. Bioactive metabolite products of *Thymus vulgaris* , Rifambin (Standard) and Cefotoxime (Standard) as anti-bacterial activity against *Proteus mirabilis*.

CONCLUSION:

- The ethanolic extract of *Thymus vulgaris* was analyzed, and it was found to contain bioactive phytochemical components. Analysis of *Thymus vulgaris* cinale using (GC-MS) uncovered the presence of the more than twenty bioactive natural compounds.
- The identification of bioactive chemical compounds is based on the peak area, retention time molecular weight and molecular formula.
- As part of the preparation for FTIR analysis, Peak (Wave number cm-^{l}), (Type of Intensity, Bond and Functional group assignment) were thirteen functional groups.
- *Thymus vulgaris* bioactive secondary metabolites were shown to show remarkable activity against *Proteus mirabilis*

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Ministry of Higher Education
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التنميط الأيضي لمركبات الأيض الثانوية باستخدام التحليل الطيفي للأشعة تحت الحمراء (FTIR) وتحويل فورييه والتحليل اللوني للغاز - قياس الطيف الكتلي (GC-MS) تقنيات المستخلص النباتي الكامل للإيثانول من *Thymus vulgaris* وتقييم نشاطه المضاد للبكتيريا

بحث علمي

قدمت إلى كلية العلوم للبنات

جامعة بابل استيفاءً جزئياً للمتطلبات

للحصول على درجة البكالوريوس في قسم الأحياء

زهراء هيثم لفتة

زهراء علي حسين

زينب حمادي حسين

Supervised by

د. عبير فوزي مراد الربيعي

2024 AD **1444 AH**

الخلاصة:

لعبت النباتات الطبية دورا أساسيا في علاج الأمراض المختلفة. تم استخدام نبات الغدة الصعترية الشائع، وهو نبات طبي، على نطاق واسع لأغراض بيولوجية وصيدلانية. مركبات الايض الثانويه هي جزيئات صغيرة تشارك في التفاعلات الأيضية، وهي ضرورية لوظيفة الخلية وصيانتها ونموها. عادة، تتراوح مركبات الايض الثانوية من 50 إلى 1500 دا، في حين أن تركيزاتها تمتد لعدة أوامر من حيث الحجم. مركبات الايض الثانوية ديناميكي للغاية، ويعتمد على الوقت، والأيضات حساسة للعديد من الظروف البيئية.

الأهداف: أهداف دراستنا: الفحص الكيميائي النباتي والتعرف على بعض المركبات المتطايرة الموجودة في المستخلص الإيثانولي الكامل لنبات الغدة الصعترية عن طريق استخدام مطيافية تحويل فورييه بالأشعة تحت الحمراء (FTIR). استخدام تقنية كروماتوغرافيا الغاز - قياس الطيف الكتلي (GC-MS) لتحديد ملامح مركبات الايض الثانوية. تقييم نشاطها المضاد للبكتيريا.

المواد والطرق: تم شراء أجزاء النبات المجففة من الأسواق المحلية لمحافظة بابل، *Thymus vulgaris*، والتي تم فحصها في مختبر النباتات المتقدم في كلية العلوم، جامعة بابل بعد التنظيف وعزل المواد الغريبة. يتم طحنها بمطحنة كهربائية، ومن ثم يتم جمع المسحوق في أكياس نايلون وحفظها في المعمل في درجة حرارة الغرفة لحين الاستخدام. تم إجراء الفصل وتحديد الهوية على GC-MS. من أجل التحضير تجريبيًا لتحليل FTIR، تم تحويل كمية صغيرة مناسبة من عينات الأوراق المسحوقة في المختبر إلى كريات باستخدام KBr. هنا تم إخضاع جميع العينات التجريبية لثلاثة اختبارات منفصلة، حيث كانت كريات KBr التي لم تتم معالجتها فعليًا بمثابة عنصر تحكم.

النتائج: كجزء من التحضير لتحليل FTIR، كانت الذروة (رقم الموجة cm^{-1})، (نوع الشدة، السندات وتعيين المجموعة الوظيفية) 663.5 (قوي، C-Cl، هاليدات الألكيل)، 687.5 (قوي، C- Cl، هاليدات الألكيل)، 870.7 (قوي، =C–H، الألكينات)، 920.9 (قوي، =C–H، الألكينات)، 1017.01 (قوي، =C–H، الألكينات)، 1047.0 (قوي، C-F، هاليدات الألكيل)، 1095.15 (قوي، C-F، هاليدات الألكيل)، 1244.17 (امتداد، C-F، هاليدات الألكيل)، 1317.00 (امتداد، C-F، هاليدات الألكيل)، 1373.19 (قوي، C-F، هاليدات الألكيل)، 1608.00 (قوي، C-F، هاليدات الألكيل)) ، 2335.50 (قوي، NH، أميد)، 1244.17 (C=C، ألكين)، 1361.12 (غير معروف)، 2358.09 (غير معروف). يعتمد تحديد المركبات الكيميائية النشطة بيولوجيًا على منطقة الذروة والوزن الجزيئي ووقت الاحتفاظ والصيغة

الجزيئية. كشف تحليل GCMS لـ *Thymus vulgaris* عن وجود Cyclohexylaminopropylamino] إيثيل فوسفات، فورميل-L-ليسين، سبيرو[2.4] هيبتان-4-واحد، 4،4-ثنائي فينيل-بوتيل)-(3فينيلبيبريدين-4، د-جلوكوز،6) --O αDgalactopyranosyl، لوسينين، 2,5-ثنائي ميثيل-4-هيدروكسي-3(H2)- فورانون، 2,3-ثنائي فينيل سيكلوبرو بيل) ميثيل فيني، حمض الدوديكانويك، 3-هيدروكسي، 2,6,10,14,18 ،22-تيتراكوسا هيكسين، 10-ميثيل-E-11-تريدسين-1-أول، إيزوشيابين ب، 3،1-ديوكسولان، 4-[[(2-ميثوكسي-4- أوكتاديسينيل)أوكسي] ميثيل] -2,2 ، -E-9-Methyl-8-tridecen-2-ol، أسيتات، E-9-Methyl-8-tridecen-2-ol، أسيتات، 5-هيدروكسي ميثيل فورفورال، 4-هيكسينال، 6-هيدروكسي-4-ميثيل-، ثنائي ميثيل أسيتال، أسيتات، α-D-جلوكوبيرانوسيد، O-α-دجلوكوبيرانوسيل، 2-سيكلوهيكسيل بيبيريدين، و3-O-ميثيل-د-جلوكوز.

تمت دراسة ما إذا كانت مركبات الايض الثانويه التي تنتجها الغدة الصعترية لها خصائص مضادة للجراثيم ضد ثلاثة مسببات الأمراض الإشريكية القولونية، المكورات العنقودية الذهبية والمتقلبة الرائعة. في البحث الحالي، تم دراسة النشاط البيولوجي للمستخلص الإيثانولي لكامل نبات الغدة الصعترية، وكذلك المضادات الحيوية التقليدية ريفامبين وسيفوتوكسيم، ضد ثلاثة مسببات الأمراض المختلفة. الإشريكية القولونية (10.04 ± 0.14، 21.77 ± 0.25، و 18.00 ± 0.21)، المكورات العنقودية الذهبية (08.71 ± 0.12، 19.02 ± 0.21، و 19.00 ± 0.21)، والمتقلبة الرائعة (12.31 ± 0.16، 22.04 ± 0.23، و 2). 3.85±0.27). أظهرت مركبات الايض الثانويه النشطة حيوياً *Thymus vulgaris* نشاطاً ملحوظاً ضد *Proteus mirabilis*، بمتوسط قيمة (12.31±0.16).

الكلمات المفتاحية: *Thymus vulgaris* ، FTIR، GCMS، المجموعات الوظيفية، مركبات الأيض الثانوية، النشاط المضاد للبكتيريا.