

Original Research Article

## Analysis of Metabolites by GC-MS and FTIR Spectroscopy Techniques to Ethanolic Whole Plant Extract from *Thymus Vulgaris* and Assessment of its Antibacterial Potency

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### Article History

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**Abstract:** **Background:** The use of medicinal plants has been crucial in the treatment of a wide range of illnesses. The medicinal herb *thymus vulgaris* has a long history of biological and pharmacological applications. Small chemicals called metabolites are involved in metabolic activities that are critical for the growth, maintenance, and function of cells. Metabolite concentrations typically span multiple orders of magnitude, and their molecular weights are typically 50–1500 Da. Metabolites are quite sensitive to a wide range of environmental factors, and the metabolome is itself very dynamic and time-dependent. **Objectives:** Our research aims were: Ethanolic whole-plant extract of *Thymus vulgaris* was analysed phytochemically and identified using Fourier Transform Infrared Spectroscopy (FTIR). Utilising the Gas Chromatography-Mass Spectrometry (GC-MS) Method for Fate Profile Analysis. Assessing Its Antimicrobial Effects. **Materials and Methods:** The dried plant parts, *Thymus vulgaris*, were sourced from the local markets of Babylon Province. After cleaning and isolating foreign substances, they were studied at the advanced Botanical laboratory at the College of Science, University of Babylon. Following their crushing by an electrical grinder, the powder was gathered in nylon bags and stored at room temperature in the laboratory until needed. A GC-MS was used for separation and identification and KBr to experimentally prepare for FTIR analysis. All experimental samples were run through three independent tests in this case, with untreated KBr pellets acting as a control. **Results:** FTIR analysis, Peak (Wave number  $\text{cm}^{-1}$ ), were 663.5 (alkyl halides), 687.5 (alkyl halides), 870.7 (Alkenes), 920.9 (Alkenes), 1017.01 (Alkenes), 1047.0 (alkyl halides), 1095.15 (alkyl halides), 1244.17 (alkyl halides), 1317.00 (alkyl halides), 1373.19 (alkyl halides), 1608.00 (alkyl halides), 2335.50 (Amide), 1244.17 (Alkene). Peak area, retention time, molecular weight, and molecular formula are the three main components used to identify bioactive chemical compounds. GCMS analysis of *Thymus vulgaris* revealed the existence: Cyclohexyl-aminopropyl-aminoethylthiophosphate, Formyl-L-lysine, Spiro-heptan-4-one, 4,4-Diphenyl-butyl-3phenyl-piperidin-4, Lucenin, 2,5-Dimethyl-hydroxy-3(2H)-furanone, 2,3-Diphenyl-cyclopropyl, Dodecanoic acid, 3-hydroxy, 2,6 -Tetracosahexaene, 10-Methyl-E-11-tridecen-1-ol, 1,3-Dioxolane, 2-methoxy-octadecenyl-oxy-methyl-2,2, 5-Hydroxy-methyl-furfural, 6-hydroxy-4-methyl-, dimethyl acetal, acetate,  $\alpha$ -D-Glucopyranoside. Researchers looked at three different bacteria—*Escherichia coli*, *Staphylococcus aureus*, and *Proteus mirabilis*—to see if the secondary metabolites made by *Thymus vulgaris* had any antibacterial effects. The present study examined the biological activity of three distinct infections using the standard antibiotics Rifampin and Cefotaxime, as well as an ethanolic extract of the whole plant *Thymus vulgaris*. *Escherichia coli* ( $10.04 \pm 0.14$ ,  $21.77 \pm 0.25$ , and  $18.00 \pm 0.21$ ), *Staphylococcus aureus* ( $08.71 \pm 0.12$ ,  $19.02 \pm 0.21$ , and  $19.00 \pm 0.21$ ), and *Proteus mirabilis* ( $12.31 \pm 0.16$ ,  $22.04 \pm 0.23$ , and  $23.85 \pm 0.27$ ). The mean value against *Proteus mirabilis* for the bioactive secondary metabolites of *Thymus vulgaris* was  $12.31 \pm 0.16$ , indicating significant efficacy.

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

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## INTRODUCTION

Throughout history, humans have found numerous uses for plants. The therapeutic efficacy of medicinal plants has prompted a plethora of research on them in the scientific community. To create novel, very powerful, safe, and financially viable medications, medicinal plants are a great place to look for lead chemicals [1]. World Health Organisation research shows that more than 80% of the global population uses medicinal plants to cure a variety of illnesses. For more than sixty thousand years, people have been utilising plants, according to fossil fuel records. The biological activities of medicinal plants, including their antibacterial, antifungal, antimalarial, and antioxidant properties, have been the subject of several investigations. Reactive oxygen species (ROS) can be generated by a variety of external sources, such as some medications [2, 3], chemicals, tobacco smoke, and stressful environmental situations. Cell damage occurs when the human body produces an excess of free radicals like  $\text{OH}^+$  and  $\text{O}_2^-$ . Lipids, deoxyribonucleic acid (DNA), and proteins are among the biologically important substances that it severely oxidatively damages. Protecting cells from damage caused by free radicals, phenolic chemicals are found in nature. Flavonoids, coumarins, curcuminoids, phenolics, lignans, tannins, and a host of other phenolic chemicals are found in many natural antioxidants. The health and food sectors have paid a lot of attention to natural antioxidants in order to identify their secondary metabolites. By removing harmful reactive oxygen species (ROS), antioxidants shield cells from free radical damage [4, 5]. Tissue damage, chemical irritation, and pathogenic infections of all kinds can trigger inflammation, a natural biological response. When pathogens induce symptoms like redness, warmth, swelling, or pain, innate immune system receptors typically set off this reaction. The Lamiaceae family includes the often used *Thymus vulgaris* in traditional medicine. Approximately 300 species [6-8], are present in the Southern Europe, Mediterranean, North Africa, and Asia regions. An important idea in the study of plant-herbivore interactions is the biochemical coevolutionary arms-race theory, which states that herbivores develop resistance mechanisms as a consequence of plant secondary metabolites evolving in reaction to herbivore pressure. It is believed that insect herbivores and plant secondary metabolites are both driven by the ensuing arms race. In recent decades, the categorisation of plant hormones, secondary metabolites, and primary metabolites has been a helpful guideline. But as our knowledge of plant metabolism grows, we may have to go back to square one with this functional partitioning. In instance, the functional trichotomy is being more and more muddled by genetic and functional investigations on secondary [9, 10], metabolites in plants, which are revealing that these compounds can act as regulators and even as precursors to primary metabolites. We go over this evidence in this review, with an emphasis on cases where this evidence is supported by chemical complementation assays and natural knockout variants, mutants, or transgenic plants that have been engineered to produce specific secondary metabolites [11]. We show that a growing number of plant secondary metabolites may not be adequately understood by keeping them functionally distinct from regulators and primary metabolites, which could impede our ability to learn how these compounds help plants survive in harsh conditions. Phytoconstituents (secondary metabolites) include phenols, tannins, glycosides, and flavonoids are abundant in *Thymus vulgaris* and are responsible for a wide range of biological activity [12-15]. This research uses gas chromatography–mass spectrometry and high-performance liquid chromatography to identify the phytochemical components of a methanolic extract of *T. vulgaris*, specifically its ethyl acetate and n-butanol fractions. A computer analysis on genes causing gastric cancer by measured phytochemicals was conducted, in addition to in vitro and in vivo biological assays for anti-inflammatory, antioxidant, haemolytic, thrombolytic, pyretic, and antidiabetic activity performed on the extracted plant. Our research aims were: Ethanolic whole-plant extract of *Thymus vulgaris* was analysed phytochemically and identified using (FTIR). Utilising the (GC-MS) method for Fate profile analysis and assessment of Its Antibacterial Effects.

## MATERIALS AND METHODS

### Preparing and Collecting Plants

The dried *Thymus vulgaris* used in this work was obtained from the marketplaces of Babylon Province. First, the team separated and cleaned the foreign materials, then examined them in the Botanical laboratory at the College of Science, University of Babylon. The samples were crushed in an electrical grinder, packed in nylon bags and put in the laboratory at room temperature until use. After rinsing the whole *Thymus vulgaris* plant with clean water, it was let to dry at room temperature for three days. I finely ground 500 g of dry plant material to get a fine powder. Powdered *T. vulgaris* was kept in sealed plastic containers up until the time it was extracted.

### Procedure for Extracting

Maceration of 500 g of dried *Thymus vulgaris* powder in 1500 mL of methanol resulted in a methanolic extract. Amorphous solid masses were produced because rotating evaporators were used for extracting and evaporating ingredients.

### Gas Chromatography-Mass Spectrometry (GC-MS)

The methanolic extract of *Thymus vulgaris* was evaluated by GC-MS using the stated protocols. Observations of the methanol extract on the GC-MS model 7890B, 5977A with 75 eV of ionisation energy were made using a DB-5MS column with a film thickness of 0.25  $\mu\text{m}$ , a diameter of 0.25 mm and a 30-meter length. One milliliter of helium was used as the carrier gas each minute. We set the MS transfer line to 280  $^{\circ}\text{C}$ , chose a split ratio of 1:6, used 1  $\mu\text{L}$  of material and

employed a 30-atomic mass unit mass scan. I went on to heat my columns to 50 °C for one minute at the beginning. I started raising the temperature 8 °C every minute until 290 °C was reached every time it was reached. A carrier gas was used to move the 1 mL of sample extract down the column [16, 17], with a helium flow rate of 1 mL/min. After purification at 75 eV in the column, the components were identified and then investigated further with FID spectroscopy. We looked at the NIST MS 2.0 libraries to get the chemical formula, molecular weight and names of these substances.

#### Fourier Transform Infrared Spectroscopy (FTIR) Analysis of *Thymus Vulgaris*

For each GLV, FTIR spectra were obtained by analysing huge amounts of data collected earlier by an FTIR instrument and executing experimental runs and computer processing. To experiment with FTIR, we crushed leaf samples, used KBr to make pellets and pressed the combination to create a thin layer before the analysis. Also, data was obtained in the wave number spectrum between 4000 cm<sup>-1</sup> and 500 cm<sup>-1</sup> to ensure reliable and sound information about how infrared light is transmitted. Three different forms of CK were tested by the method described above, with untreated KBr serving as a control.

#### Investigating How Effective Diverse Secondary Metabolites are When Used against Three Harmful Bacteria

These tests worked on the agar integrated by punching five-millimeter diameter holes using a sterile tool known as a cork borer. Three types of bacteria—*Escherichia coli*, *Staphylococcus aureus* and *Proteus mirabilis*—were afterward tested using twenty-five microliters of solutions that also contained metabolites made by *Thymus vulgaris* and the standard drugs Rifampin and Cefotaxime.

#### Statistical Analysis

We checked if our parametric data was statistically significant using Student's t-test when the p-value was less than or equal to 0.05.

## RESULTS AND DISCUSSION

Organic molecules with small weight are frequently synthesized in the form of air, water and other surroundings by living plants. In science, there are three main groups for these compounds: primary metabolites are important to plant development, secondary metabolites control plant interactions with its environment and hormones control activities inside the plant's body. For a long period, plant biology and its practical applications have followed the three-step model of plant metabolism. Exact boundaries using pure biochemical criteria could not always be determined between metabolite classes. Current research in genetics and chemistry reveals that secondary metabolites affect both development and defence in plants. Many scientists believe secondary metabolites [18, 19], need to be diverse because they are needed for reliable signaling and effectively recycled and stored. Since some herbivores change the function of plant secondary metabolites, these metabolites allow scientists to study how defence is made and help us better understand the relationships between plants and herbivores. FTIR analysis, Peak (Wave number cm<sup>-1</sup>), were 663.5 (alkyl halides), 687.5 (alkyl halides), 870.7 (Alkenes), 920.9 (Alkenes), 1017.01 (Alkenes), 1047.0 (alkyl halides), 1095.15 (alkyl halides), 1244.17 (alkyl halides), 1317.00 (alkyl halides), 1373.19 (alkyl halides), 1608.00 (alkyl halides), 2335.50 (Amide), 1244.17 (Alkene). Metabolites refer to the substances created in the process of metabolism. Almost everyone in chemistry defines metabolites to mean only small molecules. Fuel, support for reaction in cells, signalling, influencing enzymes, participation as catalyst together with enzymes, defence and interactions with different organisms are important tasks of metabolites [20-23]. Most of the organic chemicals plants make do not seem to take part in growth and development. Many plants have different amounts of these chemicals and they are commonly called secondary metabolites in science. There has recently been greater interest in secondary metabolites, mainly because they have grown in economic importance and research is now concentrating on the possible use of tissue culture to change the production of these important plant compounds. It is possible to establish cell and tissue culture routines in sterile areas using explants such as leaves, stems, roots and meristems, for both reproducing cells and extracting secondary metabolites [24, 25]. Artificial creation of secondary in a lab. Commercial medicinal herbs exhibit the presence of metabolite in their plant cell suspension cultures. Various pharmaceuticals, food additives, flavours and similar industrial materials can come from plant secondary metabolites and using plant cell cultures fixed many challenges related to obtaining them. Root cultures make secondary metabolite production better than most other methods. Secondary metabolites are biological substances that do not greatly affect an organism's normal development, growth or reproduction [26-29]. Such metabolites often appear as mixtures of close members of a chemical class, are formed only in growth's later phase, do not support growth (though they may affect survival), come from selected groups of microbes and have unusual chemical constructions. Bioactive compounds are most often identified by looking at their peak area, time of retention, molecular weight and molecular formula. GCMS analysis of *Thymus vulgaris* revealed the existence: Cyclohexyl-aminopropyl-amino]ethylthi ophosphate, Formyl-L-lysine, Spiro-heptan-4-one, 4,4-Diphenyl-butyl-3phenyl-piperidin-4, Lucenin, 2,5-Dimethyl-hydroxy-3(2H)- furanone, 2,3-Diphenyl-cyclopropyl, Dodecanoic acid, 3-hydroxy, 2,6 -Tetracos-hexaene, 10-Methyl-E-11-tridecen-1-ol, 1,3-Dioxolane , 2-methoxy- octadecenyl)oxy-methyl-2,2, 5-Hydroxy-methyl-furfural , 6-hydroxy-4-methyl-, dimethyl acetal , acetate, α-D-Glucopyranoside. To test

antibacterial effects, researchers examined three bacteria using secondary metabolites made by *Thymus vulgaris*. In the present work, the biological effect of three different types of infections was studied using two antibiotics plus an extract from the whole plant *Thymus vulgaris*. *Escherichia coli* ( $10.04 \pm 0.14$ ,  $21.77 \pm 0.25$ , and  $18.00 \pm 0.21$ ) Figure 2, *Staphylococcus aureus* ( $08.71 \pm 0.12$ ,  $19.02 \pm 0.21$ , and  $19.00 \pm 0.21$ ) Figure 3, and *Proteus mirabilis* ( $12.31 \pm 0.16$ ,  $22.04 \pm 0.23$ , and  $23.85 \pm 0.27$ ) Figure 4. *Thymus vulgaris* bioactive secondary metabolites were shown to show remarkable activity against *Proteus mirabilis*, with a mean value of ( $12.31 \pm 0.16$ ).

The way plants create important natural products called secondary metabolites is called secondary metabolism. As cells age and change in shape, the development of specific secondary metabolites seems related to the onset of morphological differentiation found during plant development. Experiments done in the lab have shown that differentiated tissues generate many more secondary metabolites than less developed tissue types. Because of these metabolites, product recovery occurs fast; challenging or costly plant cultures can be substituted with plant cells; and selecting the best cell lines makes secondary metabolite production very efficient [30, 31]. Given how quickly this field is growing, there will be many more examples to share in the future.

While metabolic engineering marks a big step forward, working only with genes won't solve the main issues that have delayed the commercial use of secondary metabolites in plants. With modern technology, plant cell cultures can provide new options for making chemicals, cells and unusual or expensive plants in commercial quantities and at a reasonable cost. It is therefore necessary to build up data from the cell and molecular level [32, 33], since our knowledge of the pathways by which plants or cultures make important substances is usually not advanced. Because the process is complicated and not well understood, scientists have worked on each case apart from one another in order to learn what has gone wrong during attempts to extract valuable chemicals from these cells. While growing secondary phytochemicals from standard plant cultures can be tricky, we are now able to obtain various useful substances from unstructured calluses or suspension. Oil from *Thymus vulgaris* is found to be less effective against gram-positive bacteria, but gram-negative bacteria are more resistant [34-36]. You can eat the flowers and leaves of *thymus vulgaris*, but thyme oil isn't safe, so don't swallow it neat and instead make a blend with a diluting oil first. It is possible for oral use of thyme oil to cause several negative effects, for example: a headache, a feeling of dizziness, low blood pressure, pain in the stomach, vomiting, diarrhoea or nausea.

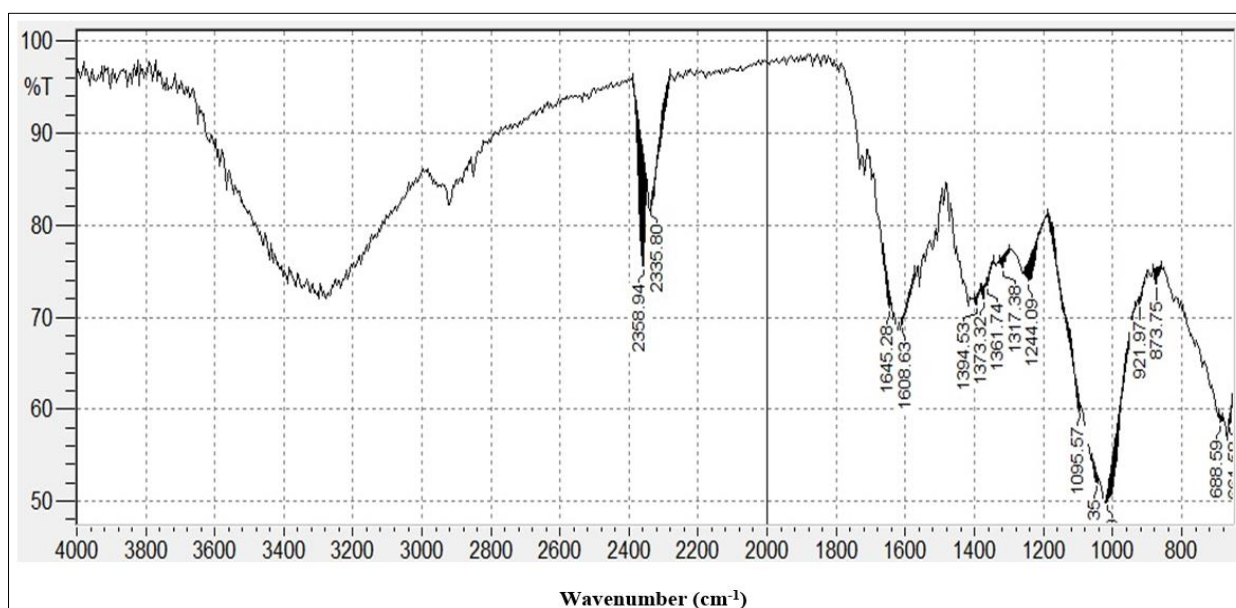


Figure 1. Fourier-transform infrared spectroscopic profile solid analysis of *Thymus vulgaris*.

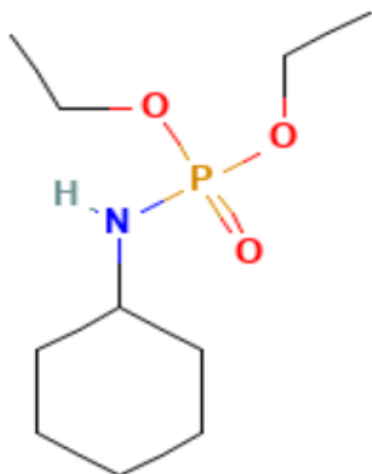


**Table 1. Fourier-transform infrared spectroscopic profile solid analysis of *Thymus vulgaris* .**

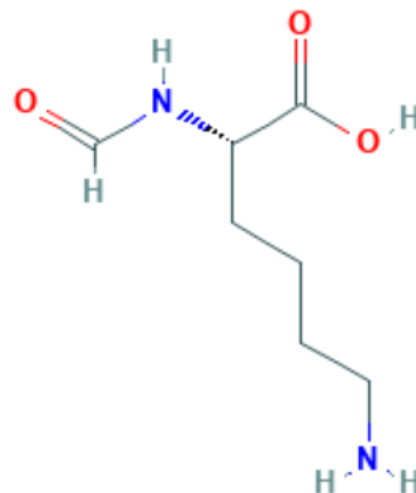
No.	Peak (Wave number cm <sup>-1</sup> )	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	663.5	58.395	0.968	663.51	653.87	2.174	0.054	Strong	C-Cl	Stretch	alkyl halides	600–800
2.	687.5	58.541	0.829	692.44	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***

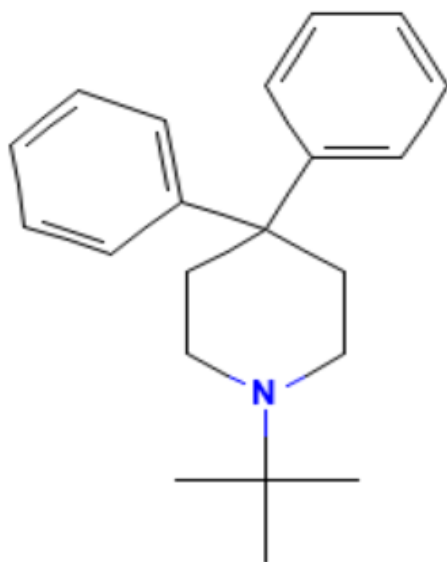
Compound	Molecular Formula	Molecular Weight t
Diethyl cyclohexylphosphoramidate	C <sub>10</sub> H <sub>22</sub> NO <sub>3</sub> P	235.26 g/mol
Formyl-L-lysine	C <sub>7</sub> H <sub>14</sub> N <sub>2</sub> O <sub>3</sub>	174.20 g/mol
1-tert-Butyl-4,4-diphenylpiperidine	C <sub>21</sub> H <sub>27</sub> N	293.4 g/mol
D-Glucose, 4-O-beta-D-galactopyranosyl	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	342.30 g/mol
Luteolin 6,8-di-C-glucoside	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	610.5 g/mol
2,5-Dimethyl-4-hydroxy-3(2H)-furanone	C <sub>6</sub> H <sub>8</sub> O <sub>3</sub>	128.13 g/mol
Phenylcyclopropyl) methanamine	C <sub>10</sub> H <sub>13</sub> N	147.22 g/mol
Dodecanoic acid, 3-hydroxypropyl ester	C <sub>15</sub> H <sub>30</sub> O <sub>3</sub>	258.40 g/mol
6,9,12,15,18,21-Tetracosahexaenoic acid,	C <sub>24</sub> H <sub>36</sub> O <sub>2</sub>	356.5 g/mol
12-Methyltridecanal	C <sub>14</sub> H <sub>28</sub> O	212.37 g/mol
Octanal, 7-methoxy-3,7-dimethyl	C <sub>11</sub> H <sub>22</sub> O <sub>2</sub>	186.29 g/mol
Methyl 4,8,12-trimethyltridecanoate	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.5 g/mol
Ethyl 2-acetyldecanoate	C <sub>14</sub> H <sub>26</sub> O <sub>3</sub>	242.35 g/mol
5-Hydroxymethylfurfural	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.11 g/mol
3-Methoxy-1-hydroxymethyl-adamantane	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	196.29 g/mol
4-O-alpha-D-Glucopyranosyl-moranoline	C <sub>12</sub> H <sub>23</sub> NO <sub>9</sub>	325.31 g/mol
2-Cyclohexylpiperidine	C <sub>11</sub> H <sub>21</sub> N	167.29 g/mol
3-O-Methyl-d-glucose	C <sub>7</sub> H <sub>14</sub> O <sub>6</sub>	194.18 g/mol



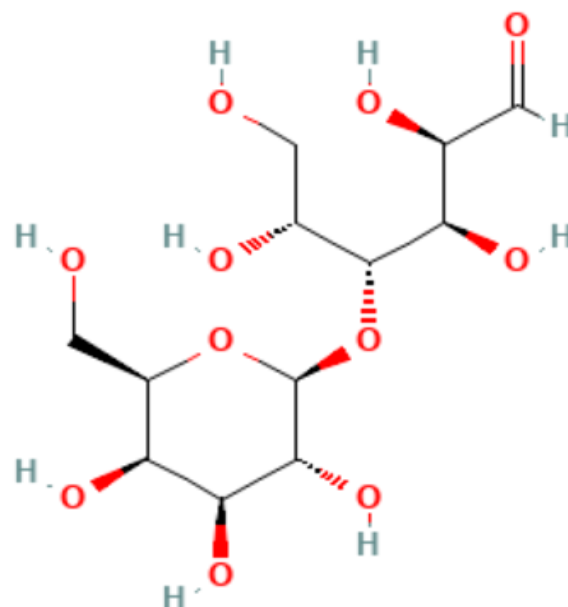
**Diethyl cyclohexylphosphoramidate**



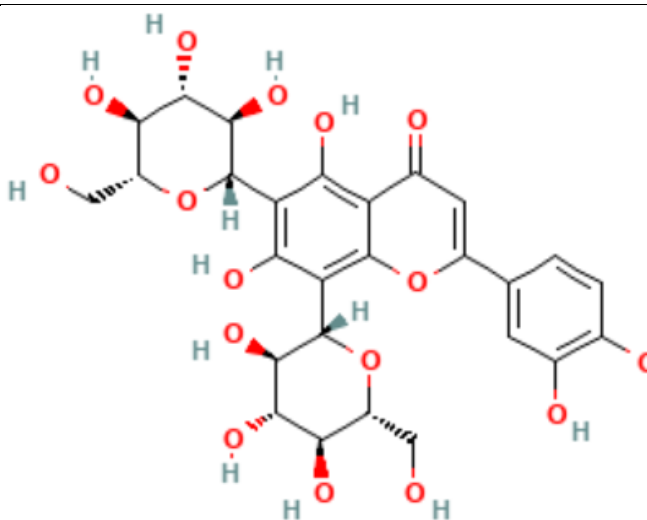
**Formyl-L-lysine**



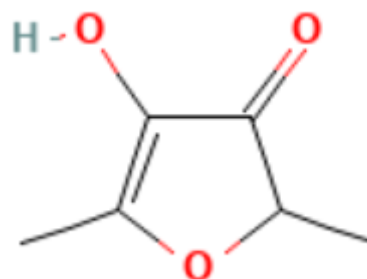
**1-tert-Butyl-4,4-diphenylpiperidine**



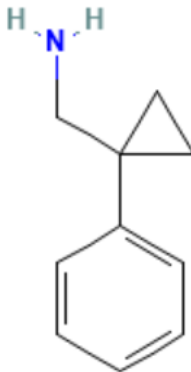
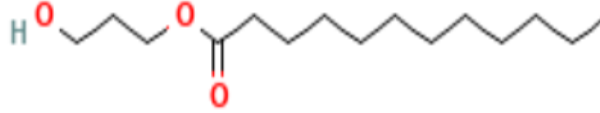
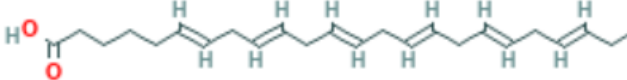
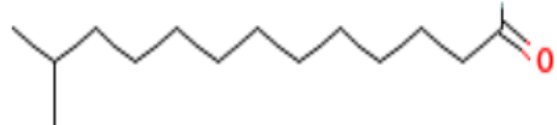
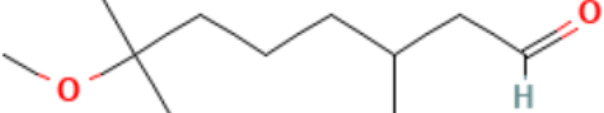
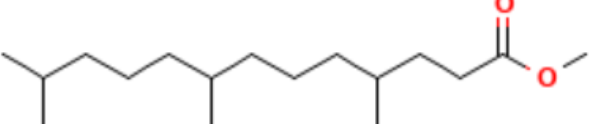
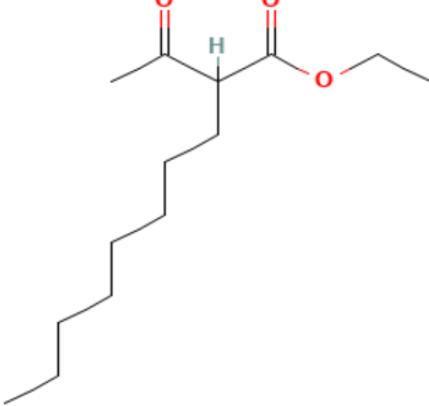
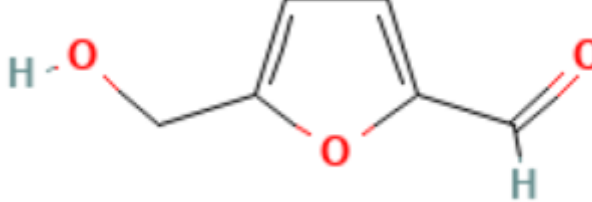
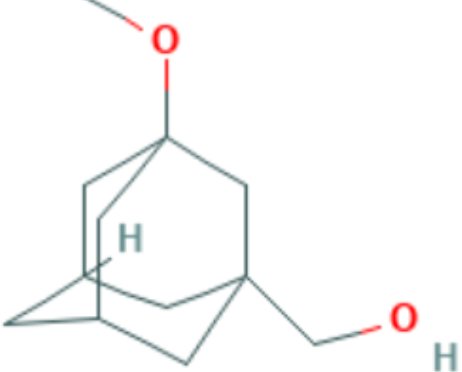
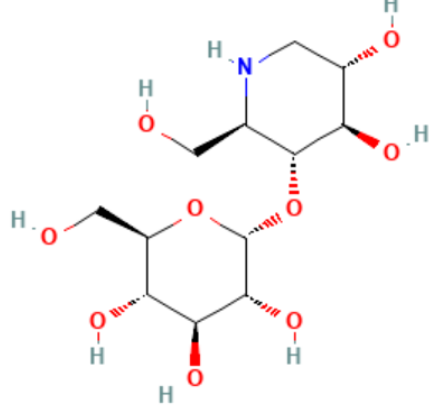
**D-Glucose, 4-O-beta-D-galactopyranosyl**

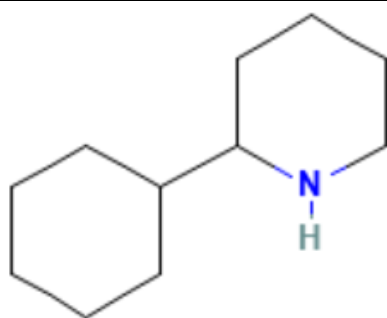


**Luteolin 6,8-di-C-glucoside**

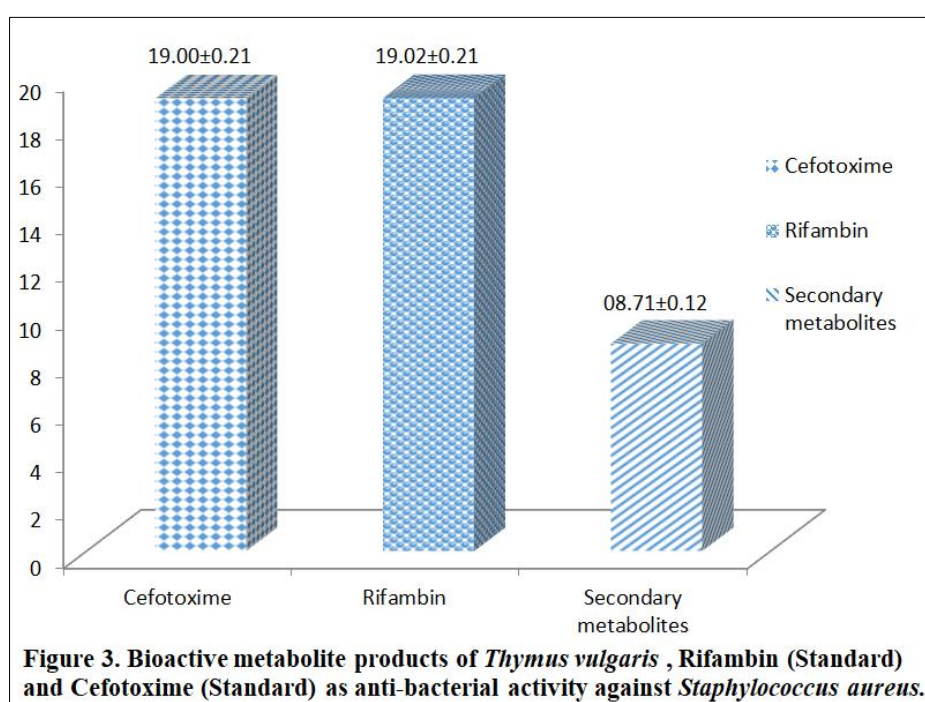
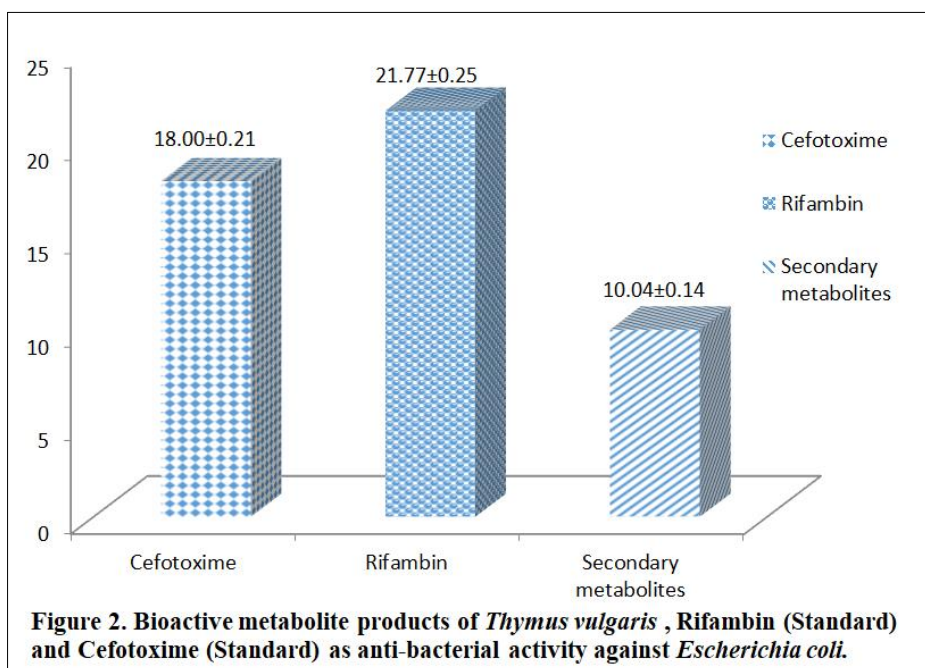


**2,5-Dimethyl-4-hydroxy-3(2H)-furanone**

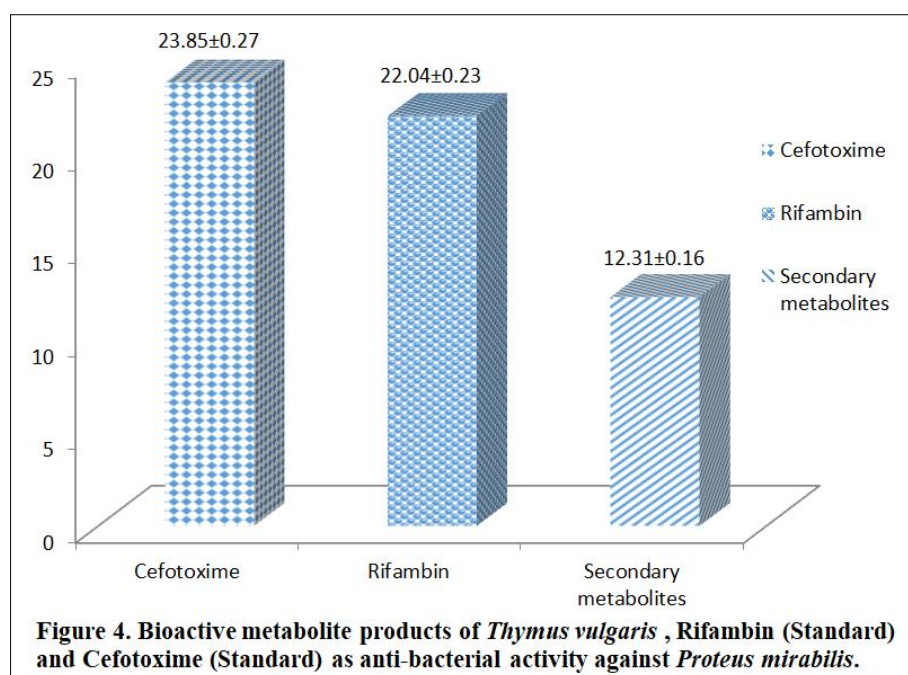
 <p><b>phenylcyclopropyl)methanamine</b></p>	 <p><b>Dodecanoic acid, 3-hydroxypropyl ester</b></p>
 <p><b>6,9,12,15,18,21-Tetracosahexaenoic acid</b></p>	 <p><b>12-Methyltridecanal</b></p>
 <p><b>Octanal, 7-methoxy-3,7-dimethyl</b></p>	 <p><b>Methyl 4,8,12-trimethyltridecanoate</b></p>
 <p><b>Ethyl 2-acetyldecanoate</b></p>	 <p><b>5-Hydroxymethylfurfural</b></p>
 <p><b>3-Methoxy-1-hydroxymethyl-adamantane</b></p>	 <p><b>4-O-alpha-D-Glucopyranosyl-moranoline</b></p>



2-Cyclohexylpiperidine







It was reported in 2009 that, when *Arabidopsis* can't make indole glucosinolate, it loses its ability to produce callose in response to Flg22, following early research on how secondary metabolites manage plant defences. Rescue of callose formation is achieved by adding 4-methoxy-indol-3-ylmethylglucosinolate. Glucosinolate breakdown in callose control is suspected due to the important role of the myrosinase PEN2. It wasn't much later that researchers discovered that, similar to secondary metabolites from indole, cereals play a critical role in regulating the plant's callose activity. The ability of aphids and chitosan to prompt callose deposition is lost in *bx1* *Zea mays* plants lacking benzoxazinoids and DIMBOA or DIMBOA-Glc applied to these mutants restores callose synthesis. In both cases, the control of callose depends on the structure of the indole-derived ring [37, 38]. The lack of a methylated hydroxy-group in its aromatic ring makes indol-3-ylmethylglucosinolate inactive in *Arabidopsis*. Although DIMBOA-Glc methylation makes it inactive in corn, the HDMBOA-Glc form is not inert. Both maize and benzoxazinoids trigger a callose response, but *Arabidopsis* does not respond to these and neither does maize to intact glucosinolates. The researchers found that secondary metabolite control over callose is very specific, closely monitored and probably developed more than once. Experts do not yet understand the process by which secondary metabolites lead to callose. As a result, glucosinolates and benzoxazinoids can edit callose formation by regulating certain hormones, guiding how genes are turned on or off or starting callose synthesis on proteins directly. It seems that some secondary metabolites are formed under the control of benzoxazinoids and glucosinolates. With both flg22 and *Pseudomonas syringae* infection, plants lacking a correct version of PEN2 produce fewer camalexin compounds. Additionally, indole glucosinolate is not present in mutants that cannot produce the sinapoylmalate created by the CYP83B1 enzyme. Too much accumulation of aldoxime may be the reason for less sinapoylmalate, as mutants short on indole-3-acetaldoxime (the substrate for CYP83B1) produce normal amounts of sinapoylmalate. Mutant versions of MEDa/b are vital to a huge transcriptional complex that controls the genes in phenylpropanoid biosynthesis. Suppressor screens revealed that these plants also lack the phenylpropanoid phenotype. A recent study has found that intake of indole glucosinolate mutants causes MED5 to increase the expression of genes in the Kelch Domain F-Box family which are implicated in activating PAL. For mutants without the glucosinolate, KFB action and accumulation of sinapoylmalate are (partially) restored. They give evidence that monooxygenation by CYP83B1 mutants leads to aldoxime buildup which upregulates MED5 and at least one more pathway to allow KFB to break down PAL [39-41]. Similar sorts of aldoximes are likely produced by diverse species which may explain why such defence regulation is not unique to glucosinolate plants. There is higher accumulation of HDMBOA-Glc and less DIMBOA-Glc in wheat lines that have increased activity of a maize O-methyl transferase. Even so, these lines have higher levels of the phenylpropanoid ferulic acid even though the concentrations of their amino acid precursors do not change. This implies that different secondary metabolite routes may control phenolic compounds.

## CONCLUSION

Analysis showed that ethanolic *Thymus vulgaris* extract contains several bioactive phytochemicals. Twenty bioactive natural chemicals were identified by a gas chromatography–mass spectrometry analysis of *Thymus vulgaris*. You can identify bioactive chemical substances by checking their peak area, retention time, molecular weight and molecular

formula. As part of preparing for FTIR analysis, thirteen functional groups were assigned, covering Peak (Wave number cm<sup>-1</sup>), Type of Intensity, Bond and Functional group assignment. Bioactive secondary metabolites from *Thymus vulgaris* showed major anti-*Proteus mirabilis* activity.

## REFERENCES

1. Ahsan, H.; Ayub, M.; Irfan, H.M.; Saleem, M.; Anjum, I.; Haider, I.; Asif, A.; Abbas, S.Q.; Ul Hussian, S.S. Tumor necrosis factor- $\alpha$ , prostaglandin-E2 and interleukin-1 $\beta$  targeted anti-arthritis potential of fluvoxamine: Drug repurposing. *Environ. Sci. Pollut. Res. Int.* 2022, 24.
2. Muhammad, I.; Xiao, Y.Z.; Hassan, S.S.U.; Xiao, X.; Yan, S.K.; Guo, Y.Q.; Ma, X.P.; Jin, H.Z. Three new guaiane-type sesquiterpenoids and a monoterpenoid from *Litsea lancilimba* Merr. *Nat. Prod. Res.* 2022, 36, 3271–3279.
3. Hassan, S.S.U.; Ali, F.; Ishaq, M.; Bano, I.; Hassan, M.; Jin, H.-Z.; Bungau, S. A Comprehensive In Silico Exploration of Pharmacological Properties, Bioactivities, Molecular docking, and Anticancer Potential of Vieloplain F from *Xylopia vielana* Targeting B-Raf Kinase. *Molecules* 2022, 27, 917.
4. Naveed, M.; Bukhari, B.; Aziz, T.; Zaib, S.; Mansoor, M.A.; Khan, A.A.; Shahzad, M.; Dablood, A.S.; Alruways, M.W.; Almalki, A.A.; et al. Green Synthesis of Silver Nanoparticles Using the Plant Extract of *Acer oblongifolium* and Study of Its Antibacterial and Antiproliferative Activity via Mathematical Approaches. *Molecules* 2022, 27, 4226.
5. Muhammad, I.; Hassan, S.S.U.; Cheung, S.; Li, X.; Wang, R.; Zhang, W.-D.; Yan, S.-K.; Zhang, Y.; Jin, H.-Z. Phytochemical study of *Ligularia subspicata* and valuation of its anti-inflammatory activity. *Fitoterapia* 2021, 148, 104800.
6. Hassan, S.S.U.; Abbas, S.Q.; Muhammad, I.; Wu, J.-J.; Yan, S.-K.; Ali, F.; Majid, M.; Jin, H.-Z.; Bungau, S. Metals-triggered compound CDPDP exhibits antiarthritic behavior by downregulating the inflammatory cytokines and modulating the oxidative storm in mice models with extensive ADMET, docking and simulation studies. *Front. Pharmacol.* 2022, 13, 1053744.
7. Naveed, M.; Batool, H.; Rehman, S.U.; Javed, A.; Makhdoom, S.I.; Aziz, T.; Mohamed, A.A.; Sameeh, M.Y.; Alruways, M.W.; Dablood, A.S.; et al. Characterization and Evaluation of the Antioxidant, Antidiabetic, Anti-Inflammatory, and Cytotoxic Activities of Silver Nanoparticles Synthesized Using *Brachychiton populneus* Leaf Extract. *Processes* 2022, 10, 1521.
8. Epstein, P.R.; Selber, J.; Borasin, S.; Foster, S.; Jobarteh, K.; Link, N.; Miranda, J.; Pomeranase, E.; Rabke-Verani, J.; Reyes, D.; et al. A life cycle analysis of its health and environmental impacts. *Oil* 2002.
9. El-Saber Batiha, G.; Magdy Beshbishy, A.; GWasef, L.; Elewa, Y.H.A.; AAl-Sagan, A.; Abd El-Hack, M.E.; Taha, A.E.; MAbd-Elhakim, Y.; Prasad Devkota, H. Chemical Constituents and Pharmacological Activities of Garlic (*Allium sativum* L.): A Review. *Nutrients* 2020, 24, 872.
10. Fernie AR, Pichersky E (2015) Focus issue on metabolism: Metabolites, metabolites everywhere. *Plant Physiol* 169: 1421–1423.
11. Neilson EH, Goodger JQD, Woodrow IE, Møller BL (2013) Plant chemical defense: At what cost? *Trends Plant Sci* 18: 250–258
12. Li J, Schuman MC, Halitschke R, Li X, Guo H, Grabe V, Hammer A, Baldwin IT (2018b) The decoration of specialized metabolites influences stylar development. *eLife* 7: e38611
13. Kliebenstein DJ, Lambrix VM, Reichelt M, Gershenzon J, Mitchell-Olds T (2001) Gene duplication in the diversification of secondary metabolism: Tandem 2-oxoglutarate-dependent dioxygenases control glucosinolate biosynthesis in *Arabidopsis*. *Plant Cell* 13: 681–693 #
14. Murphy A, Peer WA, Taiz L (2000) Regulation of auxin transport by aminopeptidases and endogenous flavonoids. *Planta* 211: 315–324
15. Pichersky E, Lewinsohn E (2011) Convergent evolution in plant specialized metabolism. *Annu Rev Plant Biol* 62: 549–566
16. Nishida R (2002) Sequestration of defensive substances from plants by *Lepidoptera*. *Annu Rev Entomol* 47: 57–92.
17. Riedlmeier M, Ghirardo A, Wenig M, Knappe C, Koch K, Georgii E, Dey S, Parker JE, Schnitzler J-P, Vlot AC (2017) Monoterpenes support systemic acquired resistance within and between plants. *Plant Cell* 29: 1440–1459
18. Rhoades DF (1983) Responses of alder and willow to attack by tent caterpillars and webworms: Evidence for pheromonal sensitivity of willows. In PA Hedin, ed, *Plant Resistance to Insects*, Vol 208. American Chemical Society, Washington DC, pp 55–68
19. Stringlis IA, Yu K, Feussner K, de Jonge R, Van Bentum S, Van Verk MC Berendsen RL, Bakker PAHM, Feussner I, Pieterse CMJ (2018) MYB72- dependent coumarin exudation shapes root microbiome assembly to promote plant health. *Proc Natl Acad Sci USA* 115: E5213–E5222
20. Zhao Z, Zhang W, Stanley BA, Assmann SM (2008) Functional proteomics of *Arabidopsis thaliana* guard cells uncovers new stomatal signaling pathways. *Plant Cell* 20: 3210–3226
21. Stahl E, Bellwon P, Huber S, Schlaeppli K, Bernsdorff F, Vallat-Michel A, Mauch F, Zeier J (2016) Regulatory and functional aspects of indolic metabolism in plant systemic acquired resistance. *Mol Plant* 9: 662–681 Stah

22. E, Hilfiker O, Reymond P (2018) Plant-arthropod interactions: Who is the winner? *Plant J* 93: 703–728
23. Zhang X, Gou M, Liu C-J (2013) Arabidopsis Kelch repeat F-box proteins regulate phenylpropanoid biosynthesis via controlling the turnover of phenylalanine ammonia-lyase. *Plant Cell* 25: 4994–5010
24. Turlings TCJ, Erb M (2018) Tritrophic interactions mediated by herbivore-induced plant volatiles: Mechanisms, ecological relevance, and application potential. *Annu Rev Entomol* 63: 433–452
25. Ugine TA, Krasnoff SB, Grebenok RJ, Behmer ST, Losey JE (2019) Prey nutrient content creates omnivores out of predators. *Ecol Lett* 22: 275–283
26. Schmelzer, G.H. and Gurib-Fakim, A (2008): Medicinal Plant 1. In: Plant resources of tropical Africa (Volume 11). Wageningen: Earthprint Limited, pp. 791.
27. Warfa AM (1988): Cordia (Boraginaceae). In: NE tropical Africa and tropical Arabia. Uppsala: Uppsala Univ., pp. 174.
28. Al-Snafi, AE (2017): The Pharmacological and therapeutic importance of Cordia myxa. *IOSR Journal of Pharmacy*, 6(6):47-57.
29. Rechinger, K.H (1997): Boraginaceae. In: Flora Iranica (Volume 48). Graz: Akademische Druck-u Verlagsanstalt, pp. 6-10.
30. Rashed, K., Luo, M.T., Zhang, L.T., and Zheng, Y.T (2015): Evaluation of anti-HIV-1 activity of Cordia myxa L., and phytochemical profile. *Banat's Journal of Biotechnology*, 5(10):75-82.
31. Pandey, B., Deshpand, B., Singh, S., and Chandrakar, V. (2014): Estimation of elemental contents of Cordia myxa and its antimicrobial activity against various pathogenic microorganisms. *Indian J. Sci. Res.*, 4(1):39-44.
32. Aberoumand A. (2011). Preliminary Evaluation of Some Phytochemical and Nutrients Constituents of Iranian Cordia myxa Fruits. *International Journal of Agricultural and Food Science* 2011; 1(2): 30-33.
33. Aberoumand, A. and Deokule, S. (2010). Assessment of the Nutritional Value of Plant-Based Diets in Relation to Human Carbohydrates: A Preliminary Study. *Advance Journal of Food Science and Technology* 2(1): 1-5.
34. Bouby, L., Bouchette, A. and Figueiral, I. (2011). Sebesten fruits (Cordia myxa L.) in Gallia Narboneusis (Southern France): a trade item from the Eastern Mediterranean?. *Veget Hist Archaeobot.* Vol. 1(20):397- 404.
35. Stamp, and Nancy "Out of the quagmire of plant defense hypotheses". *The Quarterly Review of Biology* 78 (1): 23 55.
36. Samuni-Blank, M., Izhaki, I., Dearing, MD., Gerchman, Y., Trabelcy, B., Lotan, A., Karasov, WH., Arad, Z. "Intraspecific Directed Deterrence by the Mustard Oil Bomb in a Desert Plant". *Current* 22 (13): 1218–1220,
37. Srivastava S1, Srivastava AK., —Hairy root culture for mass-production of high-value secondary metabolites. *Crit Rev Biotechnol*, 27(1):29-43. 2007 Jan-Mar
38. Smetanska II. —Production of secondary metabolites using plant cell cultures. *Adv Biochem Eng Biotechnol.* 2008; 111:187- 228.
39. A. Kossel —Über die chemische Zusammensetzung der Zelle. *Archiv für Physiologie* (1891) 181-18.
40. J.B. Harborne, N.J. Walton, D.E. Brown —Classes and functions of secondary products in chemicals from Plants, *Perspectives on Secondary plant products*. Imperial college press, pp. 1-25, 1996
41. Yukimune Y, Tabata H, Higashi Y, Hara Y —Methyl jasmonate-induced overproduction of paclitaxel and baccatin III in Taxus cell suspension cultures. *Nature Biotechnol*, 14:1129– 32, 1996.