BioChemMed

Technium BioChemMed Vol. 11, pp.128-148 (2024)
ISSN: 2734-7990
www.techniumscience.com

Phytochemical Screening, Chemical Constitutions, and Antibacterial Activity for three Medicinal Plants Against Multidrug-Resistant Bacteria

Maryam Mansoor Mathkoor

Department of Anesthesia Techniques and Intensive Care, Al Taff University College, Kerbala, Iraq

Abstract

Due to the importance of therapeutic plants in the production and preparation of drugs, The purpose of this study is to extract three medicinal plants, including seeds of S*yzygium aromaticum* "*S. aromaticum*", leaves of *Thymus vulgaris* (*T. vulgaris*) and *Myrtus communis* (*M. communis*) using the steam distillation method. It evaluated their ability to inhibit pathogenic and antibiotic-resistant bacteria, including *Staphylococcus aureus* (*S.aureus*), Pseudomonas aeruginosa (*P. aeruginosa*), and *Escherichia coli* (*E.coli*) isolated from skin ulcers. Moreover, these extracts were cured to evaluate their phytochemicals and chemical constitutions using classical methods and Gc-mass spectroscopy. The phytochemicals results showed that the Flavonoids and terpenoids were observed in all samples, while the Alkaloids didn't appear. The extracts of *S. aromaticum*, *T. vulgaris*, and *M. communis* showed interesting compounds during the GC-mass spectroscopy test. It showed the presence of chemical compounds with active groups that could have a significant role in bacterial treatment, such as 9-octadecenamide, Caryophyllene, and Limonene. Moreover, The results of the bacterial test showed a high effectiveness of these oils on the types of bacteria used, especially *S. aureus*, and *E. coli*, where the highest results were for *Myrtus* on *E. coli* bacteria with an inhibition diam of 56 mm, while clove oil did not show any effectiveness against S. aureus, the inhibition zone diameter is zero. In general, the effectiveness of these essential oils against Gram N. *P.aeruginosa* was very weak compared to the effectiveness of these essential oils on other bacterial species used in the study, where the highest diameter of inhibition of these bacteria was by clove oil and reached 15 mm.

Keywords: Antibacterial, *S. aromaticum*, *T. vulgaris*, *M. communis*, steam distillation method

Technium BioChemMed Vol. 11, pp.128-148 (2024)
ISSN: 2734-7990
www.techniumscience.com

1. Introduction

Medicinal plants have long been thought to be beneficial to one's health [1]. Herbs are still present in 40% of prescription medications [1]. Herbs are plants with fragrant qualities that are commonly employed in spicy dishes and to make herbal teas in traditional medicine [2].

Traditional medicine has used herbs and spices for so long, and they are now widely used as colourants, flavours and fragrances in the human diet. It offers chemical properties and is used in pharmaceuticals, agronomy, food, personal care, cosmetics and perfumes [3,4,5].

Essential oils (EOs) are a class of plant secondary metabolites with known antioxidant capacities that can be directly measured utilizing peroxyl radical interactions. Essential oils are fragrant, oily liquids obtained by water distillation or solvent extraction from different parts of plants, for instance, "leaves, roots, seeds, fruits, etc."[6]. On the other hand, *Thymus vulgaris L.* is an outstanding aromatic plant "with over 100 species worldwide", and is commonly used for medicinal and culinary purposes[7]. *Thyme* contains flavonoids and phenolic antioxidants such as zeaxanthin, lutein, luteolin, pigenin, naringenin, and thymonin. Among herbs, fresh thyme has one of the highest levels of antioxidants. It is considered rich in minerals and vitamins that are vital for good health. Leaves are rich in "potassium, iron, calcium, manganese, magnesium and selenium" [8]. Because of its distinct aroma, thyme essential oil is employed as a "crude material in perfumery and cosmetics" [9]. The anti-inflammatory and hepatoprotective effects of thyme essential oils were revealed[10].

True Myrtle, also known as *Myrtus communis L*., is a major aromatic and medicinal plant used in the food, pharmaceutical, and cosmetic sectors. It is a Mediterranean evergreen shrub or small tree with dense leaves that belongs to the *Myrtaceae* family [11,12]. The fruits, twigs, leaves and berries of this plant have all been used as a folk medicine to treat FBM, stomach ulcers, haemorrhage, headaches, heart palpitations, leucorrhoea, conjunctivitis, lung and cutaneous problems [13]. The essences include (linalool, geranyl acetate, 1,8-cineole, -pinene, etc.) flavonoids, glycolic acid, 1,3,7-trimethylpurine, 1-benzpyran-2-one, galloyl glucoside, ellagic acid, palmitic acid, oleic acid, linoleic acid, and stearic acid [14]. Its leaves are well-known for their essential oils, and they are also used to treat lung diseases and have antibacterial properties [15,16,17]. *M. communis* oil's pharmacological actions, including anti-inflammatory, antibacterial, antioxidant, antiviral, antifungal, and hypoglycemic characteristics, have been extensively studied [18,19].

Moreover, an important role in creating a spicy scent is played by *Clove, "Syzygium aromaticum or Eugenia aromaticum or Eugenia caryophyllata"*, a member of the *Myrtaceae* family[20]. It predominantly comprises eugenol (48-89%), -caryophyllene (5-22%), and eugenol acetate (48-89%). (0.4 - 22 percent). In addition, it contains "a minor amount of -humulene"[21]. Cloves are high in antioxidants and are efficient against bacteria, fungus, and viruses[22]. The essential oil can be extracted from buds or other plant elements. It has been revealed that clove buds contain 15–21% volatile oil [23], and 22 components of the essential oil extracted from clove buds. Eugenic acid was the most common constituent (76.23%)[24]. Clove essential oil is "antibacterial, antifungal, antiviral, anti-inflammatory, cytotoxic, insect repellent, and anaesthetic"[25,26].

Microorganisms located in adjacent areas such as *Corynebacteria* and *Propionibacteria* cause skin wound infections. Additionally, skin wound infections can also be caused by other

Technium BioChemMed Vol. 11, pp.128-148 (2024)
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www.techniumscience.com

endogenous sources or the external environment infolding multidrug-resistant bacteria such as methicillin-resistant bacteria *S. aureus* (MRSA), and Gram-negative facultative anaerobic *E. coli* and *P. aeruginosa*. It is important to note that healing is not achieved without the occurrence of bacterial growth with a generation of host immune response and tissue damage occurs [27,28]. The present study aims to extract essential oils from three medicinal plants: Thyme, Myrtus and Clove using the Clevenger apparatus. The study further aims to study the effectiveness of these oils on some types of pathogenic and antibiotic-resistant bacteria isolated from skin ulcers.

2. Material and Methods

2.1. Collection of the Plant Materials

Seeds of *'Syzygium aromaticum'* (S. *aromaticum*) and leaves of '*Thymus vulgaris'* (*T. vulgaris)* were purchased from the local markets in the holy Karbala-Iraq, while the leaves of *Myrtus communis* (*M. communis)* were collected from local farms in the same governorate. The damaged and discoloured leaves were isolated, and washed with tap water first, then with distilled water. Next, they were dried in the open air for 8 days and ground with an electric mill as well as for the other samples. Finally, all samples were stored in glass containers until use[29].

2.2. Essential Oils Extractions

After collecting all the samples needed for the study, 50g of each plant material (after grinding with an electric grinder) was put into the round bottom flask of the Clevenger Apparatus (steam distillation method), then 500 ml of distilled water and the flask was installed in the apparatus, then the beaker was installed in the apparatus and turned on. The extraction process, which took about 5 hours for each plant was carried out separately, and the largest amount of oil obtained from *Myrtus communis L*. plant was 3.5 ml[30].

2.3. Phytochemical Screening of *S. Aromaticum T. Vulgaris* and *M. Communis*

The classical methods have been used to identify the presence of alkaloids, flavonoids, terpenoids, tannins, steroids, glycosides, phenols, and saponins in the extract of *S. aromaticum*, *T. vulgaris* and *M. communis*.

2.3.1 Test of Alkaloids

The *S. aromaticum*, *T. vulgaris* and *M. communis* extracts were solubilized in 100 mL of distilled water. Then they were filtered, and exposed to steam with 2 mL filtrate and 3 drops of 1% HCl. Subsequently, 1 mL of the animated liquid was amalgamated with 6 mL of the Mayer-Wagner reagent. The occurrence of alkaloids is signified by the appearance of "a cream or brown-red precipitate" [31].

2.3.2 Test of Flavonoids

200 mg of the plant extracts of *S. aromaticum*, *T. vulgaris* and *M. communis* were combined with 10 ml of ethanol and subsequently filtered. Moreover, 2ml of the filtrate was mixed with strong hydrochloric acid and magnesium ribbon. The emergence of a "pink or red hue signifies the existence of flavonoids" [31].

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2.3.3 Test of Terpenoid

The existence of terpenoids was ascertained in the extracts by the observed reddish-brown hue in the terpenoid test, which involved the combination of "0.5 mL of each crude extract with 2 mL of chloroform and 3 mL of sulphuric acid" [31].

2.3.4 Test of Tannins

Approximately 200 mg of extracts of *S. aromaticum, T. vulgaris,* and *M. communis* were heated with distilled water (10 mL). Ferric chloride (0.1%) was added to the distilled water, and the combination was subsequently examined for blue-black colouration, an indication of tannins' presence [31].

2.3.5 Test of Steroids

A combination of crude extract (around 1 mL), chloroform (10 mL), and sulphuric acid (10 mL) resulted in the creation of a bilayer "a red upper layer and a greenish lower layer), indicating the presence of steroids" [32].

2.3.6 Test of Glycosides

The addition of 1 mL of distilled water and NaOH to 0.5 mL of each crude extract resulted in a yellowish colouration, indicating the presence of glycosides [32].

2.3.7 Test of Phenols

Approximately 1 mL of each extract was mixed with three drops of FeCl3 and 1 mL of K2Fe(CN)6. The emergence of greenish-blue forms validated the existence of phenols [32].

2.3.8 Test of Saponins

0.5 ml of the extract of *S. aromaticum, T. vulgaris, and M. communis* were combined with 5 ml of distilled water and agitated. The formation of foam indicated the presence of saponins [32].

2.4 Gc- mass spectroscopy of *S. aromaticum, T. vulgaris, and M. communis*

The crude extracts of S. aromaticum, T. vulgaris, and M. communis were cured to evaluate the volatile compounds that would be present in them.

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2.3. Culture Conditions and Strains

Three clinical isolates of pathogenic bacteria (*P. aeruginosa*, *S. aureus* and *E. coli*) were isolated at the Department of Health's Karbala Institute of Public Health and diagnosed by VITEK 2. The bacterial strains were aerobically grown on nutrient agar (NA) plates (Hi-Media) at 37 °C for 24 h. Bacteria were activated by culturing in broth liquid medium in a shaking incubator (Labotech) at 100 rpm for 24 hours before the antibacterial activity test [33].

2.4. Antibacterial Essential Oil Activity Test

The present study employed an agar-well diffusion technique to investigate the antibacterial activity of essential oils. Mueller Hinton Agar (MHA) Hi-Media was produced, autoclaved, and cooled to 45 degrees Celsius before being put into sterile Petri dishes. The inoculum was applied to MHA plates with a sterile cotton swab that had been soaked with the pathogenic bacterial suspension and allowed to dry. Each plate has one 5-mm-diameter well drilled into the agar surface. For each isolate, 3 agar plates were plated, the first well receiving 50 µl *Thymus vulgaris* and the second well receiving 50 µl *Myrtus communis*. Her third well in the dish received 50 µl of *Syzygium aromaticum*. Also, the researcher incubated the plates for 24 hours at 37°C. Finally, to evaluate the essential oil activity, the study measured the diameter of the growth inhibition zone of pathogenic strains [34].

3. Results

The phytochemical screening of these three crude extracts has been evaluated to present chemical groups, i.e., alkaloids, flavonoids, terpenoids, tannins, steroids, glycosides, phenols, and saponins. The results showed Flavonoids and terpenoids in all of them, while Alkaloids didn't appear, as illustrated below in (Table 1). Based on the results, all three plants must have an excellent ability to be used as an antibacterial due to the presence of these significant chemical groups, which promise to be used in the biomedical field. Moreover, the present study used antibacterial evaluation to confirm that.

Table 1: The phytochemical screening of *S. aromaticum, T. vulgaris, and M. communis*

No	Test	*S. aromaticum*	*T. vulgaris*	*M. communis*
1	Alkaloids	-	-	-
2	Flavonoids	+	+	+
3	Terpenoid	+	+	+
4	Tannins	+	-	+
5	Steroids	+	+	-
6	Glycosides	+	-	+
7	Phenols	-	+	+
8	Saponins	+	-	+

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Table 2 shows twenty-four compounds were identified to exist in the extract of *S. aromaticum*, and three compounds were observed with lower percentages named S1, S2 and S6; their percentages were 0.21%, 0.07% and 0.72%, respectively. Moreover, four higher percentages of compounds were observed and identified as S11, S15, S17 and S22, with 7.11%, 9.81%, 12.44% and 8.91% in their percentage, respectively.

Table 2: GC-mass spectroscopy of *S. aromaticum*

NO	Compounds name	Area%	RT
1	Caryophyllene oxide	0.21	8.289
2	Geraniol	0.07	8.8117
3	Terpinen-4-ol	1.25	9.765
4	1,2-Cyclopentanedione	2.52	10.0032
5	5-Hydroxyazouracil	1.09	12.8901
6	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone	0.72	12.977
7	2,2-Diethyl-3-methyl-1,3-oxazolidine	3.52	13.0346
8	4 h-1-benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5- dihydroxy-7-methoxy	4.58	13.535
9	Hexadecanoic acid	1.34	13.7098
10	2,2-dideutero octadecanal	5.89	13.9705
11	9-Octadecenamide	7.11	14.4901
12	-β-Pinene	1.28	14.579
13	Butanedioic acid	5.76	15.2396
14	Linalool	6.94	16.0554
15	Caryophyllene	9.81	16.357
16	Methyl eugenol	7.19	16.7012
17	Elemol	12.44	17.8141
18	β-bisabolene	1.36	17.9001
19	Alloaromadendrene	1.02	17.9879
20	1 1,2-Bis(trimethylsilyl)benzene	3.93	18.0382
21	Hexanol	4.65	18.2946
22	Camphene	8.91	18.4677
23	Terpinolene	2.13	18.8243
24	Caryophyllene oxide	4.98	19.0501

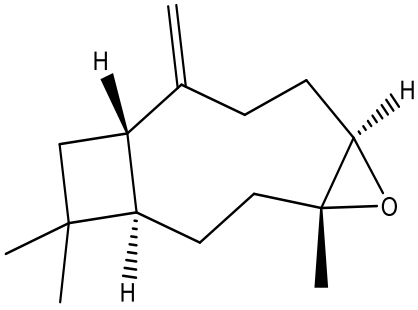
Table 3 indicates the physical and chemical properties of the obtained compounds. The data were analysed using ChemDraw software to determine their properties.

Table 3: Physical and chemical properties of identified compounds of *S. aromaticum*

No	Chemical Formula	Exact Mass	Molecular Weight	Elemental Analysis	Compounds number
1	C15H24O	220.18	220.36	C, 81.76; H, 10.98; O, 7.26	**1S**
2	C10H18O	154.14	154.25	C, 77.87; H, 11.76; O, 10.37	**2S**
3	C10H18O	154.14	154.25	C, 77.87; H, 11.76; O, 10.37	**3S**
4	C5H6O2	98.04	98.10	C, 61.22; H, 6.17; O, 32.62	**4S**
5	C8H6N6O5	266.04	266.17	C, 36.10; H, 2.27; N, 31.57; O, 30.05	**5S**
6	C6H8O4	144.04	144.13	C, 50.00; H, 5.60; O, 44.40	**6S**
7	C8H17NO	143.13	143.23	C, 67.09; H, 11.96; N, 9.78; O, 11.17	**7S**
8	C18H18O7	346.11	346.34	C, 62.42; H, 5.24; O, 32.34	**8S**

9	C16H32O2	256.24	256.43	: C, 74.94; H, 12.58; O, 12.48	**9S**
10	C18H34D2O	270.29	270.50	C, 79.93; H, 14.16; O, 5.91	**10S**
11	C18H35NO	281.27	281.48	C, 76.81; H, 12.53; N, 4.98; O, 5.68	**11S**
12	C10H16	136.13	136.24	C, 88.16; H, 11.84	**12S**
13	C4H6O4	118.03	118.09	C, 40.68; H, 5.12; O, 54.19	**13S**
14	C10H18O	154.14	154.25	C, 77.87; H, 11.76; O, 10.37	**14S**
15	C15H24	204.19	204.36	C, 88.16; H, 11.84	**15S**
16	C11H14O2	178.10	178.23	C, 74.13; H, 7.92; O, 17.95	**16S**
17	C15H26O	222.20	222.37	C, 81.02; H, 11.79; O, 7.19	**17S**
18	C15H24	204.19	204.36	C, 88.16; H, 11.84	**18S**
19	C15H24	204.19	204.36	C, 88.16; H, 11.84	**19S**
20	C12H22Si2	222.13	222.48	C, 64.78; H, 9.97; Si, 25.25	**20S**
21	C6H14O	102.10	102.18	C, 70.53; H, 13.81; O, 15.66	**21S**
22	C10H16	136.13	136.24	C, 88.16; H, 11.84	**22S**
23	C10H16	136.13	136.24	C, 88.16; H, 11.84	**23S**
24	C15H24O	220.18	220.36	C, 81.76; H, 10.98; O, 7.26	**24S**

The obtained compounds were drawn using ChemDraw software, and they showed promising chemical groups such as hydroxyl (OH), carboxyl (COOH) and amino (NH2) groups and some interesting elements, as shown in Figure 1.



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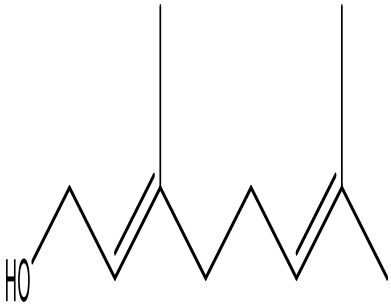

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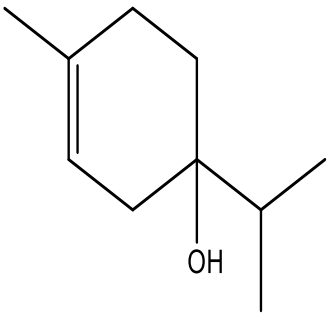

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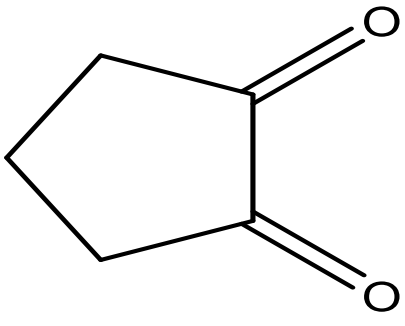

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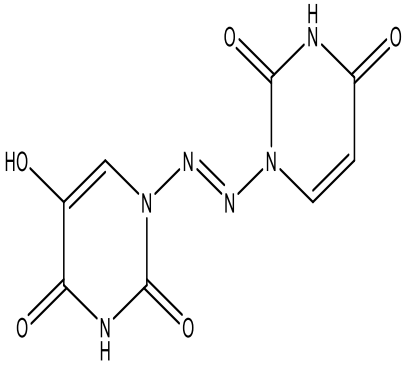
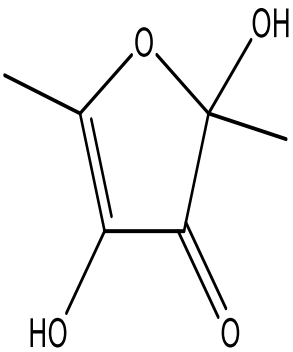


5S



6S

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ISSN: 2734-7990
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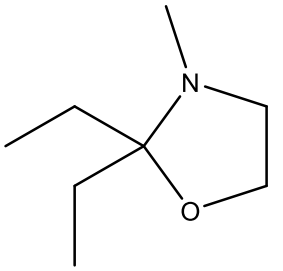

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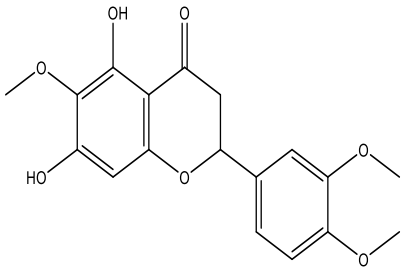

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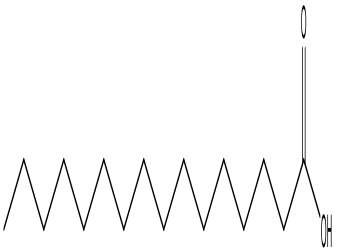

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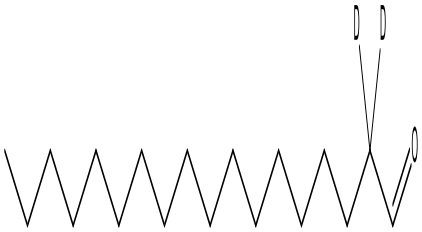

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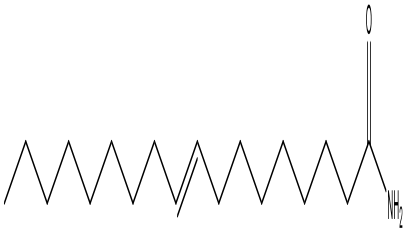

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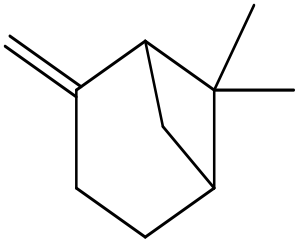

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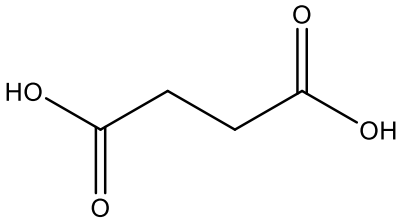

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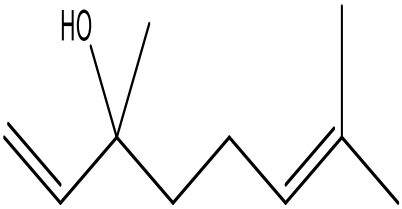

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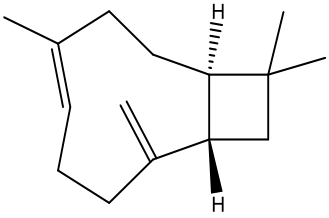

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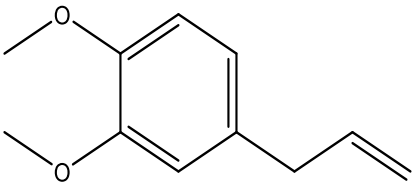

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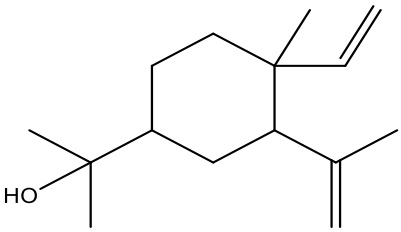

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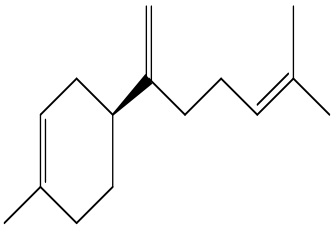

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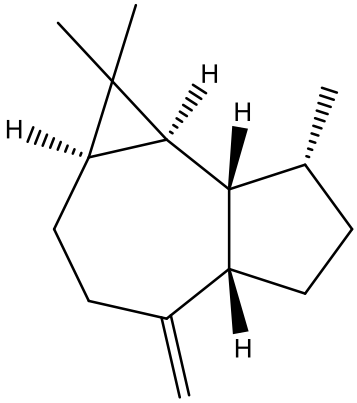

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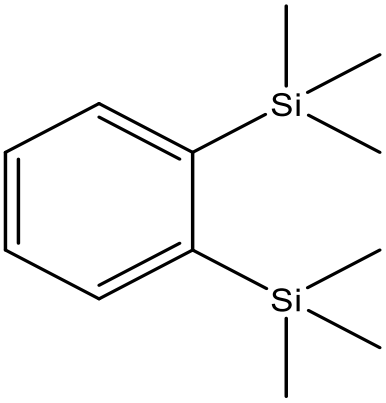

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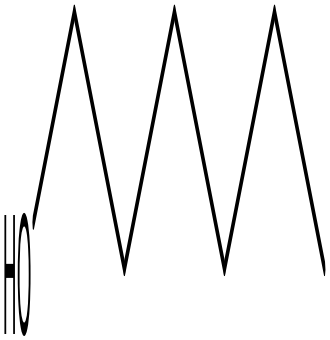

21S

Technium BioChemMed Vol. 11, pp.128-148 (2024)
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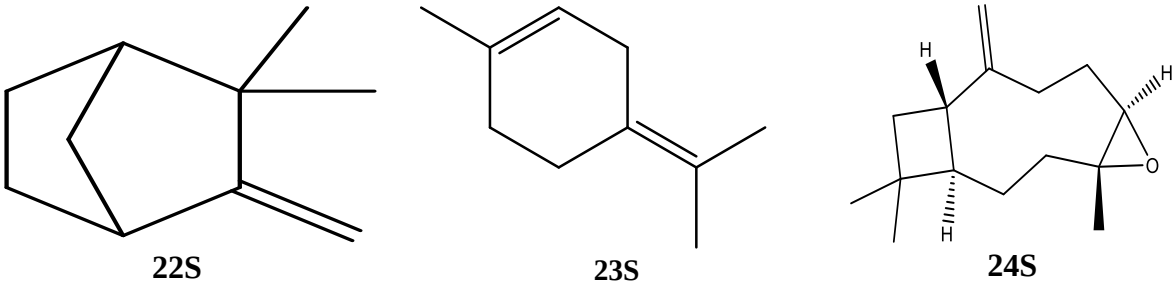

22S **23S** **24S**

Figure 1: The chemical structure of the obtained compounds from S. aromaticum

The crude extract of *T. vulgaris* was evaluated for its available compounds using GC-mass spectroscopy, and the nineteenth compounds were identified, as explained in Table 4. Three compounds were identified with low percentages, named 3T, 6T and 9T, with 0.95%, 0.21% and 0.78% in their percentages, respectively. Moreover, three compounds appeared with higher percentages, including 5T, 13T, and 15T, with 4.56%, 8.42%, and 6.73%, in their percentages, respectively.

Table 4: GC-mass spectroscopy of *T. vulgaris*

NO	Compounds name	Area%	RT
1	β-pinene	1.22	6.5031
2	Caryophyllene	1.25	6.613
3	Aromadendrene	0.95	6.912
4	α-Humulene	3.91	7.4626
5	Neryl acetate	4.56	7.7031
6	Thymol Methyl Ether	0.21	8.0243
7	Acetophenone	2.43	8.149
8	2-Ethyl-3- trimethylsilyl oxy(TriMet hylsilyl)butyrate	1.85	8.5612
9	2- Pentamethyldisilanylox pentane	0.78	8.8913
10	o-Cymene	2.03	9.192
11	Limonene	2.54	9.283
12	Carvacrol methyl ether	3.67	9.7127
13	Caryophyllene	8.42	9.9364
14	Germacrene	3.19	10.481
15	α-Murolene	1.36	10.5923
16	m-Cymene	2.81	10.9612
17	Ethyl-2-octanoate	1.94	11.4923
18	3,7-Dimethyl-1,6-octadien-3-ol	6.73	13.8915
19	Geranyl acetate	1.35	14.6719

As is indicated below, in Table 5, ChemDraw software was used to determine the properties, physical and chemical, of the attained compounds. Moreover, the properties of those compounds showed promising results.

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ISSN: 2734-7990
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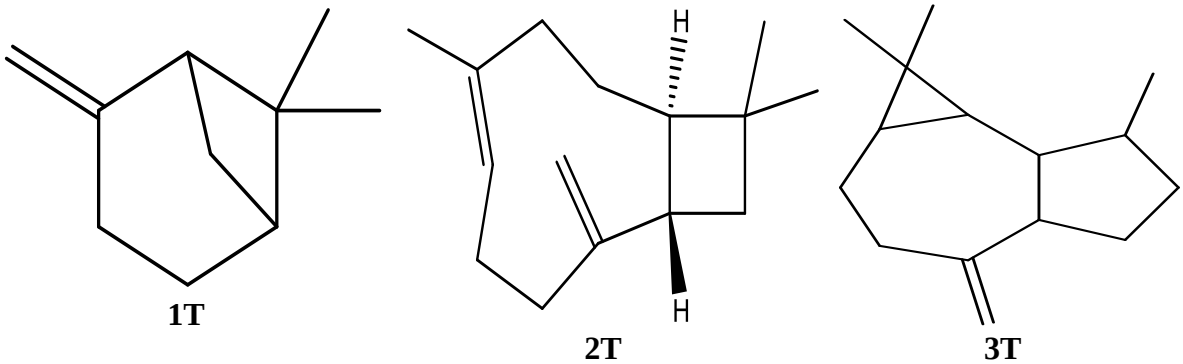
Table 5: Physical and chemical properties of identified compounds of *T. vulgaris*

No	Chemical Formula	Exact Mass	Molecular Weight	Elemental Analysis	Compounds number
1	C9H16	124.13	124.23	C, 87.02; H, 12.98	**1T**
2	C15H24	204.19	204.36	C, 88.16; H, 11.84	**2T**
3	C15H24	204.19	204.36	C, 88.16; H, 11.84	**3T**
4	C15H24	204.19	204.36	C, 88.16; H, 11.84	**4T**
5	C9H14O	138.10	139.11	C, 78.21; H, 10.21; O, 11.58	**5T**
6	C10H18O	154.14	154.25	C, 77.87; H, 11.76; O, 10.37	**6T**
7	C10H16	136.13	136.24	C, 88.16; H, 11.84	**7T**
8	C10H16	136.13	136.24	C, 88.16; H, 11.84	**8T**
9	C10H16O	152.12	152.24	C, 78.90; H, 10.59; O, 10.51	**9T**
10	C12H22Si2	222.13	222.48	C, 64.78; H, 9.97; Si, 25.25	**10T**
11	C19H15N	257.12	257.34	C, 88.68; H, 5.88; N, 5.44	**11T**
12	C11H16O	164.12	164.25	C, 80.44; H, 9.82; O, 9.74	**12T**
13	C10H14O	150.10	150.22	C, 79.96; H, 9.39; O, 10.65	**13T**
14	C15H28	208.22	208.39	C, 86.46; H, 13.54	**14T**
15	C15H24	204.19	204.36	C, 88.16; H, 11.84	**15T**
16	C10H14	134.11	134.22	C, 89.49; H, 10.51	**16T**
17	C10H15O2-	167.11	167.23	C, 71.82; H, 9.04; O, 19.13	**17T**
18	C10H18O	154.14	154.25	C, 77.87; H, 11.76; O, 10.37	**18T**
19	C12H20O2	196.15	196.29	C, 73.43; H, 10.27; O, 16.30	**19T**

The chemical structures of the compounds of the identified compounds were drawn using ChemDraw software, and they were observed to have significant chemical groups, including OH, COOH and NH2, as shown in Figure 2.



1T **2T** **3T**

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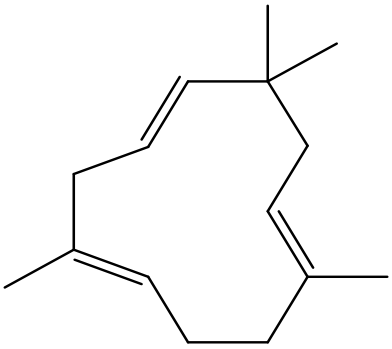

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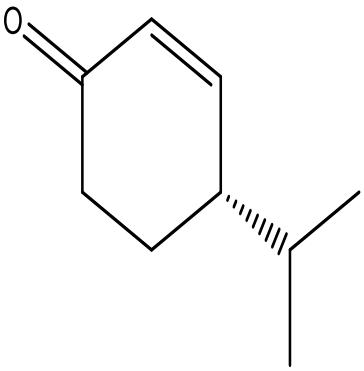

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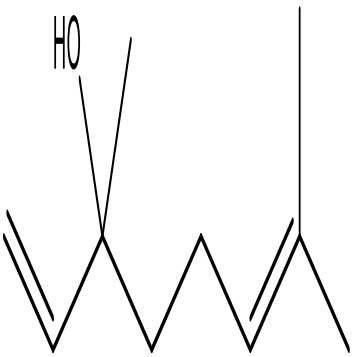

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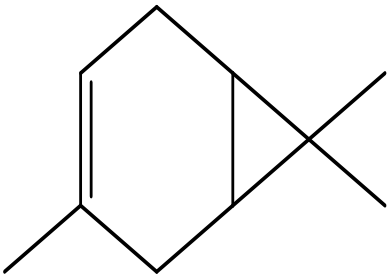

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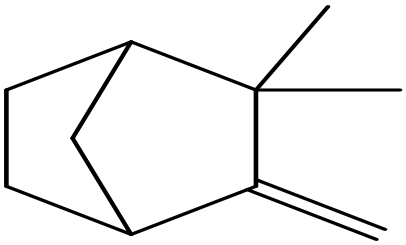

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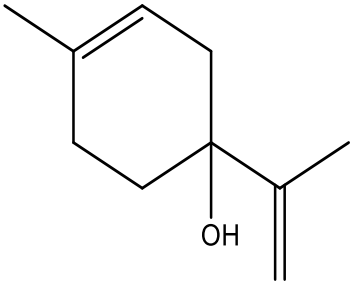

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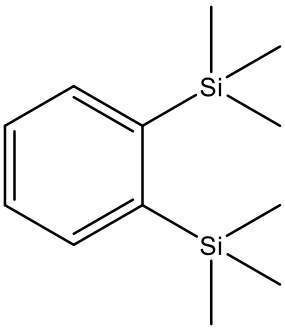

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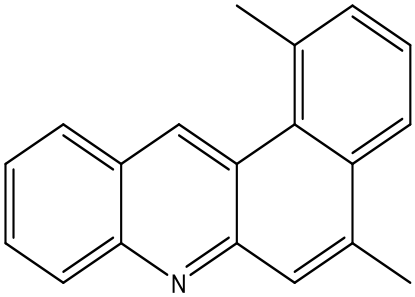

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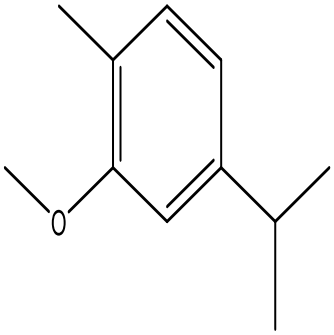

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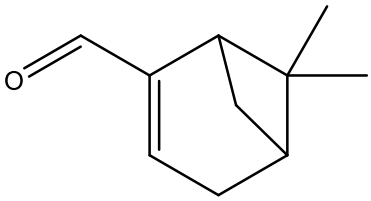

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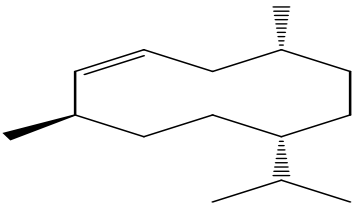

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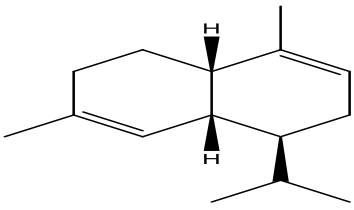

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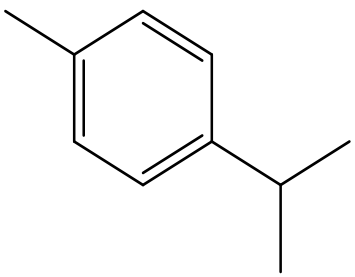

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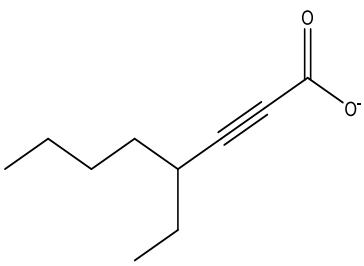

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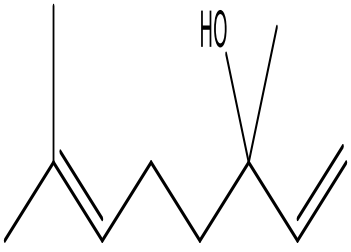

18T

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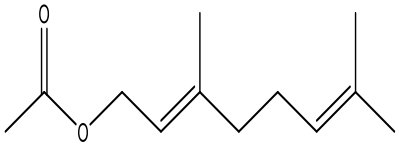


19T

Figure 2: The chemical structure of the obtained compounds from *T. vulgaris*

In the crude extracts of Myrtus communis, the study recognized 22 compounds four of which were observed with lower percentages named 4M, 9M, 13M, and 16M with 0.89%, 0.05%, 0.62%, and 0.92% in their percentages. However, three compounds were identified with higher percentages, named 8M, 14M, and 18M, with 4.35%, 3.84%, and 4.95% in their percentages, as shown in Table 6.

Table 6: GC-mass spectroscopy of *Myrtus communis*

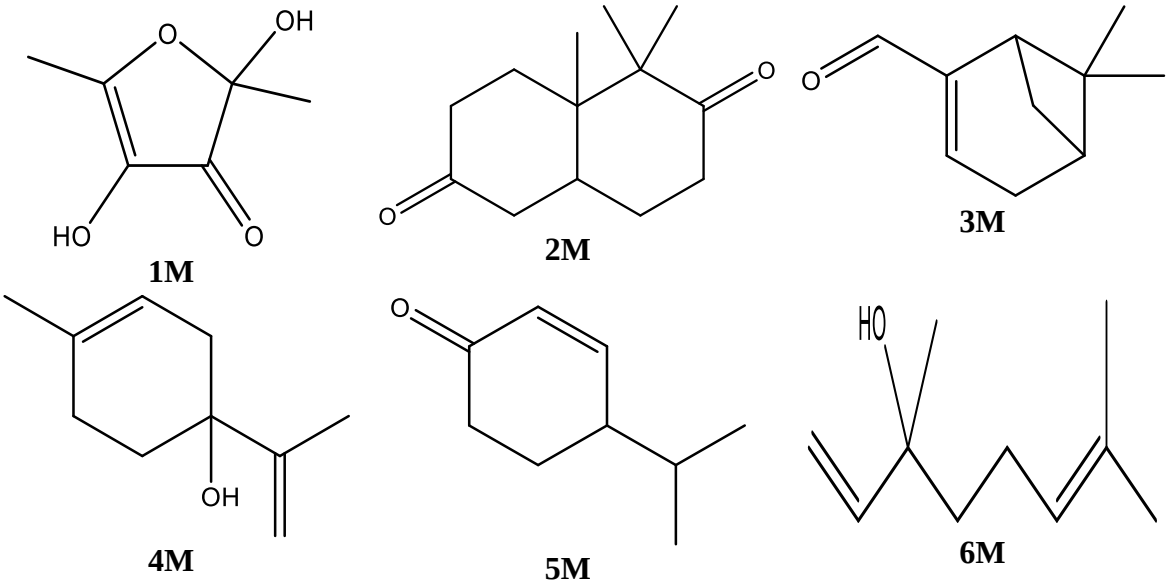
NO	Compounds name	Area%	RT
1	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone	1.44	7.7614
2	1,1,8a-Trimethyloctahydro-2,6-naphthalene dione	1.23	7.8879
3	Myrtenal	1.02	7.8879
4	1,8-Menthadien-4-ol	0.89	7.9932
5	4-ısopropylcyclohex-2-en-1-one	2.1	8.0134
6	Linalool	1.78	8.1993
7	3-Carene	3.56	8.4267
8	Camphene	4.35	8.6223
9	1,8-Menthadien-4-ol	0.05	8.8538
10	1 1,2-Bis(trimethylsilyl)benzene	1.34	9.4793
11	Benz[a]acridine, 1,5-dimethyl	1.22	10.3652
12	N-Methyl-1-adamantaneacetamide	1.69	10.5896
13	Benzamide, 2-fluoro-N-(hept-2-yl)	0.62	10.7185
14	Geranyl acetate	3.84	11.6781
15	α-terpinyl acetate	2.41	12.537
16	cis-Sabinene hydrate	0.92	13.4812
17	1,8-Cineole	2.37	14.7919
18	Limonene	4.95	15.2963
19	Myrcene	1.05	16.7417
20	β-Phellandrene	1.45	17.8126
21	δ-3-Caren	2.34	18.9431
22	Caryophyllene oxide	1.58	19.4798

Table 7 shows the chemical and physical properties of the obtained compounds, which can be observed in the ability of these compounds to be used in the biomedical field.

Table 7: Physical and chemical properties of identified compounds of *Myrtus communis*

No	Chemical Formula	Exact Mass	Molecular Weight	Elemental Analysis	Compounds number
1	C6H8O4	144.04	144.13	C, 50.00; H, 5.60; O, 44.40	**1M**
2	C13H20O2	208.15	208.30	C, 74.96; H, 9.68; O, 15.36	**2M**
3	C10H14O	150.10	150.22	C, 79.96; H, 9.39; O, 10.65	**3M**
4	C10H16O	152.12	152.24	C, 78.90; H, 10.59; O, 10.51	**4M**
5	C9H14O	138.10	138.21	C, 78.21; H, 10.21; O, 11.58	**5M**
6	C10H18O	154.14	154.25	C, 77.87; H, 11.76; O, 10.37	**6M**
7	C10H16	136.13	136.24	C, 88.16; H, 11.84	**7M**
8	C10H16	136.13	136.24	C, 88.16; H, 11.84	**8M**
9	C10H16O	152.12	152.24	C, 78.90; H, 10.59; O, 10.51	**9M**
10	C12H22Si2	222.13	222.48	C, 64.78; H, 9.97; Si, 25.25	**10M**
11	C13H21NO	207.16	207.32	C, 75.32; H, 10.21; N, 6.76; O, 7.72	**11M**
12	C14H20FNO	237.15	237.32	C, 70.86; H, 8.49; F, 8.01; N, 5.90; O, 6.74	**12M**
13	C12H20O2	196.15	196.29	C, 73.43; H, 10.27; O, 16.30	**13M**
14	C12H20O2	196.15	196.29	C, 73.43; H, 10.27; O, 16.30	**14M**
15	C10H18O	154.14	154.25	C, 77.87; H, 11.76; O, 10.37	**15M**
16	C10H18O	154.14	154.25	C, 77.87; H, 11.76; O, 10.37	**16M**
17	C10H16	136.13	36.24	C, 88.16; H, 11.84	**17M**
18	C10H16	136.13	136.24	C, 88.16; H, 11.84	**18M**
19	C10H16	136.13	136.24	C, 88.16; H, 11.84	**19M**
20	C10H16	136.13	136.24	C, 88.16; H, 11.84	**20M**
21	C10H16	136.13	136.24	C, 88.16; H, 11.84	**21M**
22	C15H24O	220.18	220.36	C, 81.76; H, 10.98; O, 7.26	**22M**

The obtained compounds from the crude extract of Myrtus communis were drawn using ChemDraw software, and their structure showed promising chemical groups such as COOH, COH, OH, and NH2, as shown in Fig 3.



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ISSN: 2734-7990
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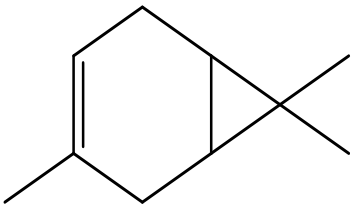

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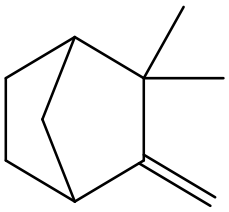

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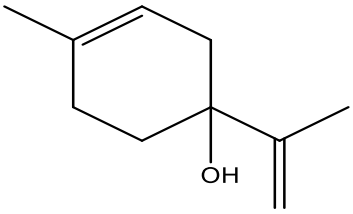

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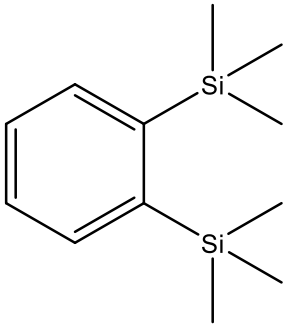

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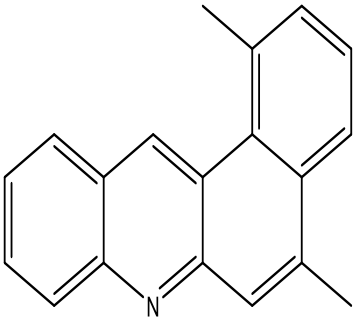

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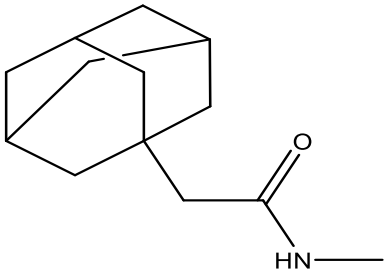

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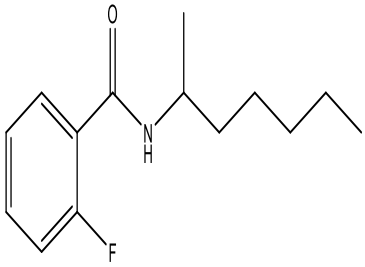

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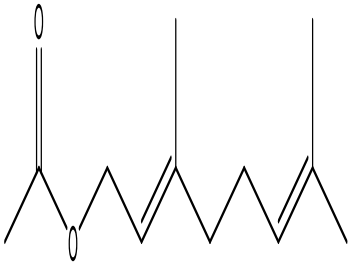

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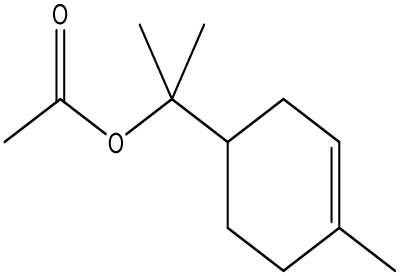

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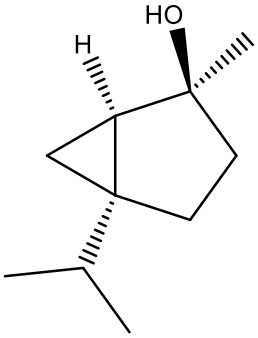

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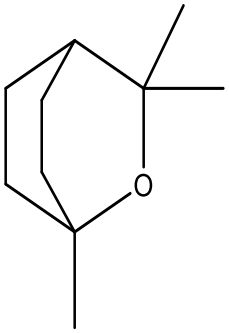

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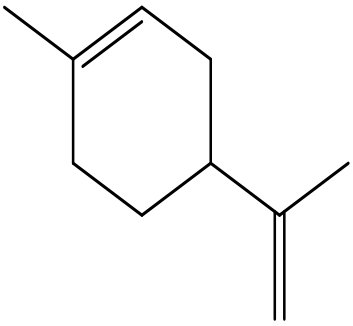

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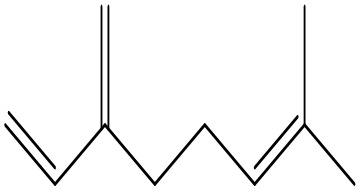

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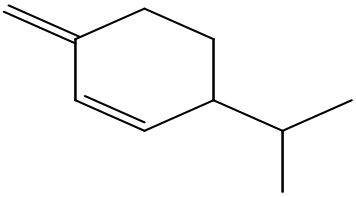

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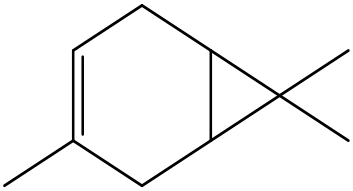

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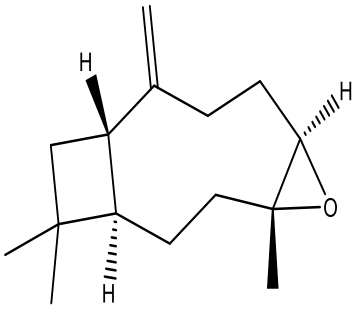


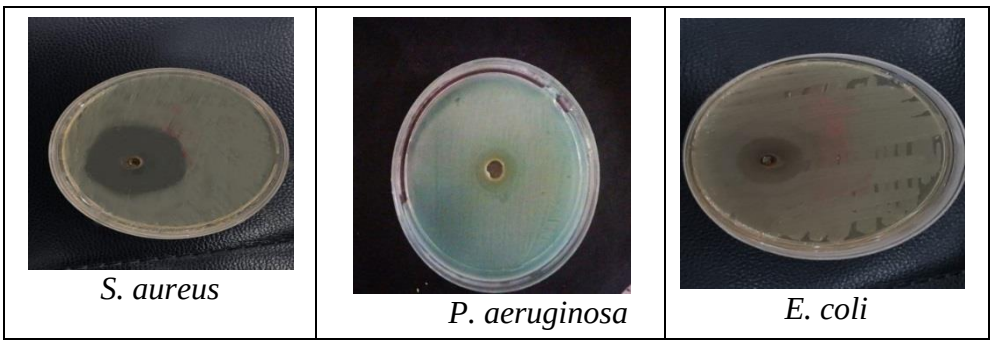
22M

Figure 3: The chemical structure of obtained compounds from *Myrtus communis*

The antibacterial Comparison in (IZD) (mm) by *S. aromaticum, T. vulgaris, and M. communis* on *S. aureus, P. aeruginosa* and *E. coli* at 37 ° C for 24 h.

Table 8: Antibacterial activity of *S. aromaticum, T. vulgaris, and M. communis*

Bacteria / Antibiotic	*S. aureus*	*P. aeruginosa*	*E. coli*
T. vulgaris	41	10	25
M. communis	40	11	56
S. aromaticum	0	15	30
Gentamicin (CN30)	0	0	0
Vancomycin (VA5)	25	0	0
Gentamicin (GEN10)	0	24	19



S. aureus *P. aeruginosa* *E. coli*

Figure 4: Antibacterial activity of *Thymus vulgaris L.* oil

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ISSN: 2734-7990
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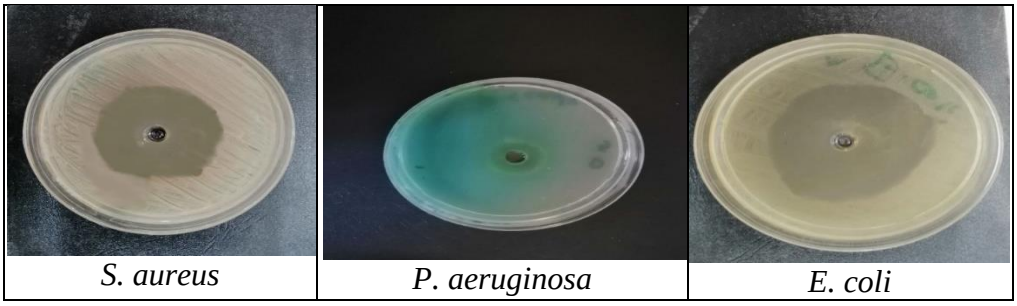

Figure 5: Antibacterial activity of *Myrtus communis L.* oil

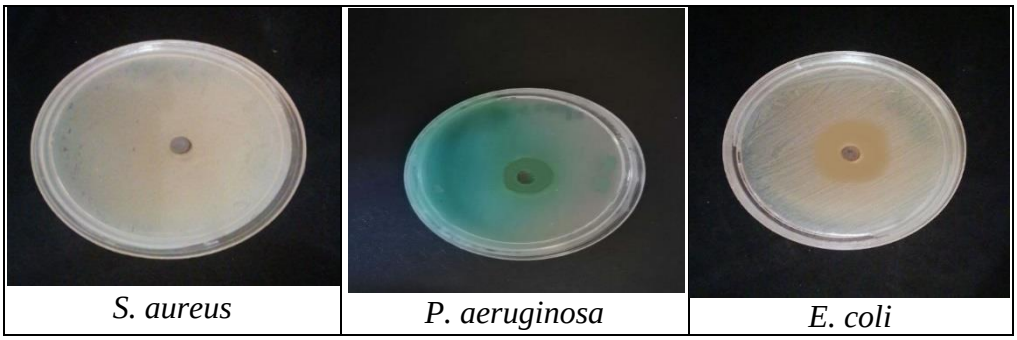

Figure 6: Antibacterial activity of *Syzygium aromaticum* oil

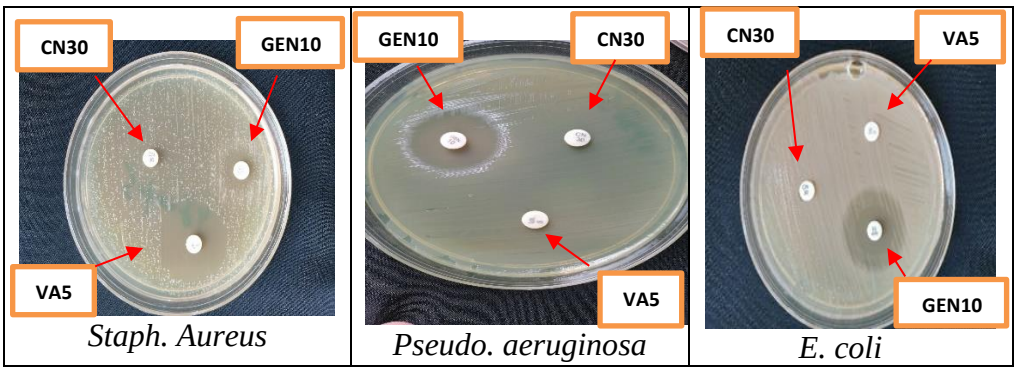

Figure 7: "Antibacterial Activity of Antibiotics"

4. Discussion

The current study was conducted to extract three medicinal plants and evaluate their chemical compositions and their biological properties. Two parts of the results were obtained: firstly, the chemical constituents were evaluated using classical methods to determine the chemical of the groups of the compounds and using GC Mass to evaluate the compounds that possibly present. Secondly, the biological properties were evaluated using antibacterial.

As mentioned in the results section Table 1, the extracts of *S. aromaticum, T. vulgaris, and M. communis* showed the presence of flavonoids and terpenoids, while Alkaloids didn't appear. This can explain its excellent ability to be used as an antibacterial due to the presence

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ISSN: 2734-7990
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of these significant chemical groups, which promise to be used in the biomedical field. Moreover, the present study used antibacterial evaluation to confirm that.

A similar study was conducted to extract *S. aromaticum* and evaluate the presence of chemical groups of the compounds. The results were shown in the presence of flavonoids, glucose, steroids, terpenoids, phenolic compounds, saponins, and tannins, which are compatible with the current results[(35)]. Moreover, another study was also reported to evaluate the phytochemicals of *S. aromaticum,* and the alkaloids did not appear similar to the present study[(36)].

The GC Mass spectroscopy of the extracts of *S. aromaticum, T. vulgaris*, and *M. communis* shows to present significant compounds with promising percentages, i.e. S11, S15 and S17 in extract *S. aromaticum*, and 5T, 13T, and 15T in*T.Vulgaris* and 8M, 14M, and 18M in M. communis, as shown in Tables 2, 4 and 6.

The highest percentage of the compound was S17, which appeared in the extract of S. aromaticum. Based on previous studies, these plants were shown priming compounds. For instance, the study conducted by Wang [(37)] was reported to evaluate the GC mass spectroscopy of S. aromaticum, and he reported to present monoterpenes and esters as a high percentage which have significant functional groups, which is in agreement with the present study.

It is noted from Table 8 that the inhibition diameters of oils against bacteria are relatively high The main reason for this is that these oils contain chemical compounds that are highly effective in inhibiting bacterial growth.

In this study, the essential oils of three medicinal plants, namely *Thyme*, *Myrtus* and *Cloves*, were extracted and used to inhibit 3 types of bacteria that cause dermatitis and are resistant to antibiotics: *P.aeruginosa*, *S. aureus* and *E. coli.* The least effect of this oil on *P. aeruginosa* bacteria with an inhibition zone diameter of 10 mm, While the inhibition zone diameter of *E.coli* bacteria was 25 mm, The highest effect of thyme oil was on *S. aureus* bacteria, with an inhibition zone diameter of 41 mm (fig.4), It is the highest inhibition of these bacteria compared to other oils, *Thyme* oil contains both antibacterial: thymol and carvacrol Both these monoterpenes are biosynthesized by the hydroxylation of p-cymene after the aromatization of γ-terpinene to p-cymene[38,39].

Oil *Myrtus communis L*. showed very high activity in inhibiting Gram-negative bacteria where the inhibition zone diameter was 56 mm on *E.coli* bacteria and 40 mm in *S. aureus* while the inhibition zone diameter of pseudo gram-negative is only 11 mm (fig.5), Most of the studies conducted on *Myrtus communis L*. oil showed the activity of this oil as an anti-bacterial like: *E. coli, Staph. Aureus, Strep. pneumoniae, Strept. pyogenes, Listeria monocytogenes, Proteus vulgaris, Pseudo. aeruginosa* and *Helicobacter pylori*[40,41] .The reason for this is due to that it contains approximately fifty composite of active compounds as anti-bacterial: 4-terpineol, α-pinene, limonene, 1,8-cineole, α-terpineol, linalool, geranyl acetate, δ-3-Carene, methyl eugenol, phenolic, β–Myrcene, α –Phellandrene and ace[42,43,44]

Clove oil showed high effectiveness on *E.coli bacteria* 30mm, While this oil did not show any activity in inhibiting *S. aureus* bacteria, *Clove* oil achieved the highest inhibition zone diameter of *P.aeruginosa* bacteria 15mm (fig.6). *Clove* oil contains a high percentage of eugenol which plays an important role in the activity of *clove* oil as an antibacterial. Eugenol and phenolic compounds in *Clove* oil damage proteins and react with phospholipids in cell membranes to alter permeability and inhibit numerous Gram-negative and G-positive bacteria. [45,46,47].

Clove extract has antimicrobial activity against a variety of bacteria, including *P. aeruginosa, Listeria, S. typhi, B. cereus, E.coli,* and *Enterococcus*. *Clove* extract has a specific

Technium BioChemMed Vol. 11, pp.128-148 (2024)
ISSN: 2734-7990
www.techniumscience.com

inhibitory effect on biofilms, studies have shown that clove extract reduces biofilm formation by up to 99% [48,49].

In general, it is noted that the highest inhibition zone diameter obtained is for *Myrtus communis L*. oil against *E.Coli* bacteria, with an inhibition diameter of 56 mm, While *S. aureus* bacteria was not affected by *Clove* oil, the inhibition zone diameter for this bacteria was zero mm.

If we compare the effectiveness of oils with the three types of antibiotics used in the study (fig.7), We will notice that the highest inhibition was for a Vancomycin (VA5) on *S.aureus* bacteria with a diameter of 25mm and This antibiotic had no effect on the other two types of bacteria.

While the effect of Gentamicin (GEN10) was on two types of bacteria, The highest effect was on *P.aeruginosa* bacteria with a diameter inhibition of 24mm (While the effect of Gentamicin (GEN10) on two types of bacteria, The highest effect was on *Pseudomonas aeruginosa* bacteria with a diameter inhibition of 24mm), The effect of this antibiotic on *Escherichia coli* was 19mm While it did not affect *S. aureus* bacteria.
Gentamicin (CN30) antibiotic did not affect any type of bacteria.

5. Conclusions:

The present study aimed to extract three therapeutic herbs and assess their chemical compositions and biological activities. Two components of the findings were acquired: first, the chemical contents were evaluated by conventional techniques to identify the chemical groups of the compounds, and subsequently, GC Mass was used to analyse the potentially present compounds. Secondly, the biological features were assessed using antibacterial evaluation. Based on the results, it was concluded that *Myrtus communis* oil gave the highest inhibition zone diameter compared to other oils, and this activity was against *E.coli*, *Clove* oil did not show any activity in inhibiting gram-positive Staphylococcus aureus bacteria, while the highest result was in inhibiting *E.coli* bacteria. The study also concludes that all extracted oils didn't show high activity in inhibiting *Pseudomonas* bacteria compared to the other types of bacteria used in the research.

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