

GC-MS analysis of essential oils of *Humulus lupulus*, *Malva Sylvestris* and *Thymus* plants in water solvent

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ABSTRACT

New extraction methods, such as ultrasonic and microwave extraction, are fast and effective methods for extracting effective compounds. From plant tissues. Ultrasound waves are soundwaves with frequencies above 20 kHz that create mechanical oscillations in a solid, liquid or gas in this study, the response surface method was used to determine the optimal points of the extraction process by ultrasonic method to achieve maximum phenolic compounds and extract efficiency and the effect of factors such as frequency and temperature on the extraction of phenolic compounds, extract efficiency and anti- activity. In this method, we extracted the extracts of three medicinal plants *Humulus lupulus*, *Malva sylvestris* and *Thymus* in a water. Then, using GC-MS device, we investigated their chemical components. In *Humulus lupulus*, the maximum percentage efficiency with 31.15% is related to 1,2- Benzenedicarboxylic acid and in *Malva sylvestris* and *Thymus* plants, it is related to 46.23% respectively and 10.53% is related to Dimethylphosphinic acid and Methyl .beta-d-galactopyranoside.

Keywords: *Humulus lupulus*, *Malva Sylvestris*, *Thymus*, phenilic, ultrasonic.

Introduction

Cancer is one of the lethal diseases it is characterized via the irregular cell proliferation. The very common reason for cancer is lifestyle changes, therefore there is urgent requirement to discover a better treatment for this lethal disease (According to World Health Organization) ^[1]. High fatality and incidence implicit it as a significant public health and economical issue that requires an effective way for prevention. Medicinal plants owns many advantages than chemical products because the compounds that are derived from plants are more tolerant with no toxic effect to the normal cells of the human body ^[2]. Several pharmacological roles of these plants compounds includes antioxidant, antimicrobial, antiviral, anticancer, antifungal and

anti-parasitic roles ^[3]. The available classic curatives for cancer therapy are radiotherapy and chemotherapy both have diverse side effects such as neurological effect, cardiac effect, renal and pulmonary toxicity effect sorely affecting the healthiness of the person. Thus a substitutional method for treatment is in demand that include development of anticancer drug that is less toxic and more potent as compare to the already available drugs in the market. Many studies have been done on naturally occurring plant compounds known to own cytotoxicity effects as they demonstrate potentiality to destroy cancerous cells. On account of the advantages of medicinal plants they are on top of demand and various species have been scanned and selected for the accommodation of cancer medicines. Newly, there has been an elevated interest in the study of compounds from a plant source as anticancer compounds by the concerned scientists. Many studies have showed the role of these plants in prevention and therapy of cancer ^[4]. presently, near 25% of the drugs produced in all around the world are extracted from plants in a direct way or at least one of its active ingredient is from plant origin, According to world health organization 80% of the world's population depend on plants derived drugs for treatment ^[5-8], Also WHO promotes the herbal derived drugs addition in health care programs because these drugs are easy to access at a low

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cost within the reach of the common people and are time tested, thus counted to be much safer comparing to up to date synthetic drugs [9-12]. Malva sylvestris is one of the medicinal herbs that used in both food and medicine, this plant characterized by a perennial root, a juicy annual stem of 2-3 height, large heart-shaped seven-lobed leaves, and the Flowers is closely similar to that of honeysuckle [13-15]. Nowadays, the consumption of this plant is broad spread because modern researches have revealed the important medicinal properties of this plant such as; anti-ulcerogenic, antioxidant, anticancer, and anti-inflammatory [16, 17].

Materials and Methods

Extract extraction methods:

Ultrasound method:

In this method, 20 g of medicinal plants including Humulus lupulus, Malva sylvestris, Thymus were weighed with a balance to the nearest 0.001 g and by electric blade mill (Moulinex MC 300.32 food)

It was ground for one minute and passed through a 16 sieve and mixed with 100 ml in a water and by creating ultrasonic waves in this study from an ultrasound tube device (MPI, Switzerland) with power Production of 1000 watts and frequency of \pm 20 kHz and a cylindrical Ti-shaped probe with a diameter of 20 mm were used. The temperature was kept constant during the extraction angle using a water bath and a thermometer was used to control the temperature. The extract was centrifuged at 3200 rpm for 45 minutes and then the clear top of the mixture was separated. The solvent was applied by lottery at 40 ° C to prevent damage to the phenolic compounds at about 100 rpm. The concentrated extract was recorded on a plate and placed inside at 40 ° C while the extraction circuit dried completely. Then the plate lid was placed and closed around it with paranium, and to prevent light penetration, it was completely covered with aluminum foil and the phenolic compounds were kept at -180 ° C.

Gas chromatography and mass spectrometry (GC/MS)

GC/MS analysis were carried out in Jahad e Daneshgahi of Urmia, in West Azerbaijan Provinc. The chemical composition of essential oils Humulus lupulus, Malva Sylvestris and Thymus were analyzed by gas chromatography-mass spectrometry (GC/MS) using a gas chromatograph equipped with a flame ionization detector (FID) and with PH-5 capillary column (30 m \times 0.1 mm; 0.25 μ m film thickness). The oven temperature was held at 60 ° C for 3 min, programmed at 20 ° C/ min to 240 ° C and then held at this temperature for 8.5 minute. The carrier gas was Helium (99.99 %) at a flow rate of 1 ml/min. Mass spectra were taken at 70 eV. The injector temperature was 280 ° C. Identification of the constituents of the oil was made by

comparison of their mass spectra and Retention Indices (RI) with those given in the literature and those authentic samples. Chemical compounds of essential oils include esters, aldehydes, alcohols, phenols, ketones and terpenes. Computer matching identified compounds used as references. The relative concentrations of the separated compounds based on percentage were computed from chromatograms obtained with the GC/FID/MS system.

GC-MS analysis Extraction of Humulus lupulus with water:

In the extraction method of Humulus lupulus with water in the ultrasound device, 40 chemical components with different percentage efficiencies were identified. The percentage and type of compounds identified were analyzed by GC-MS. The chemical compounds observed in each of the medicinal plants were different because the medicinal plants have different chemical compounds in them after extraction and analysis. The following are 40 chemical compounds observed in the presence of this medicinal plant with different efficiency percentages, which are as follows:

In peak number 1 at RT= 4.899 with a purity of 1.54 % is related to Acetic acid. In peak number 2 at RT=5.752 with a purity of 1.17 % is related to 3,3-Dimethylcyclopentanone. In peak number 3 at RT=11.033 with a purity of 1.25 % is related to Hexane. In peak number 4 at RT= 11.508 with a purity of 4.08 % is related to Propane. In peak number 5 at RT= 12.263 with a purity of 0.90 % is related to 4a.beta.-methyl-2,3,4,4a,7,8-hexah. In peak number 6 at RT=13.156 with a purity of 1.33 % is related to Thymol. In peak number 7 at RT=13.385 with a purity of 1.71 % is related to phenol. In peak number 8 at RT=13.871 with a purity of 0.90 % is related to Acetic acid. In peak number 9 at RT=13.917 with a purity of 0.89 % is related to Acetic acid. In peak number 10 at RT= 15.451 with a purity of 1.72 % is related to Tetradecane. In peak number 11 at RT=15.679 with a purity of 1.44 % is related to Octadecanal. In peak number 12 at RT=17.459 with a purity of 1.24 % is related to Undecane. In peak number 13 at RT=17.528 with a purity of 2.91 % is related to Trisiloxane. In peak number 14 at RT=17.814 with a purity of 3.66 % is related to phenol. In peak number 15 at RT= 18.191 with a purity of 0.74 % is related to 2-Octyn-1-ol. In peak number 16 at RT=18.352 with a purity of 0.85 % is related to Heptane. In peak number 17 at RT=19.490 with a purity of 2.48 % is related to Nonadecane. In peak number 18 at RT=19.656 with a purity of 0.64 % is related to (+)-2,3-O-Ethoxymethylene-d-ribose lactone. In peak number 19 at RT= 19.782 with a purity of 1.29 % is related to Permethylated. In peak number 20 at RT= 19.891 with a purity of 2.79 % is related to 2-Oxabicyclodec-9-en-8-one. In peak number 21 at RT=20.034 with a purity of 0.59 % is related to beta.-D-Glucopyranoside. In peak number 22 at RT=20.160 with a purity of 1.62 % is related to tetrahydro geranyl acetate. In peak number 23 at RT= 23.46 with a purity

of 0.75 % is related to 3-Cyclohexene-1-ethanol. In peak number 24 at RT= 20.709 with a purity of 7.20 % is related to Alloocimene. In peak number 25 at RT= 20.864 with a purity of 7.36 % is related to Ethanone. In peak number 26 at RT=21.081 with a purity of 5.74 % is related to Hydroxydihydroedulan. In peak number 27 at RT=21.602 with a purity of 1.24 % is related to Octacosane. In peak number 28 at RT= 23.547 with a purity of 1.37 % is related to 1-Heptadecanamine. In peak number 29 at RT= 24.205 with a purity of 0.68 % is related to d-Gala-l-ido-octitol Silane. In peak number 30 at RT= 24.960 with a purity of 0.77 % is related to Didodecyl phthalate. In peak number 31 at RT= 25.315 with a purity of 0.62 % is related to Octane. In peak number 32 at RT= 25.424 with a purity of 0.89 % is related to Pentatriacontane. In peak number 33 at RT= 25.859 with a purity of 1.73 % is related to Heneicosanoic acid. In peak number 34 at RT= 26.471 with a purity of 0.80 % is related to Hexadecane. In peak number 35 at RT= 26.854 with a purity of 0.83 % is related to Heptadecanoic acid. In peak number 36 at RT= 28.222 with a purity of 0.65 % is related to 1,1- ideuterio-hexadecanyl methane sulfonated.

In peak number 37 at RT= 28.508 with a purity of 0.68 % is related to Octadecane. In peak number 38 at RT=28.617 with a purity of 0.75 % is related to Heneicosane. In peak number 39 at RT= 29.246 with a purity of 1.05 % is related to Heptadecanoic acid. In peak number 40 at RT= 32.822 with a purity of 31.15 % is related to 1,2-Benzenedicarboxylic acid.

Table 1. Humulus lupulus essential oil constituents in water

Peak No.	Compound Name	R.T.	Area %
1	Acetic acid	4.899	1.54
2	3-Dimethylcyclopentanone	5.752	1.17
3	Hexane	11.033	1.25
4	Propane	11.508	4.08
5	4a.beta.-methyl-2,3,4,4a,7,8-hexahydro-5(6H)-naphthalenone	12.263	0.90
6	Thymol	13.156	1.33
7	Phenol	13.385	1.71
8	Acetic acid	13.871	0.90
9	Acetic acid	13.917	0.89
10	Tetradecane	15.451	1.72
11	Octadecanal	15.679	1.44
12	Undecane	17.459	1.24

13	Trisiloxane	17.528	2.91
14	Phenol	17.814	3.66
15	2-Octyn-1-ol	18.191	0.74
16	Heptane	18.352	0.85
17	Nonadecane	19.490	2.48
18	(+)-2,3-O-Ethoxymethylene-d-ribofuranose lactone	19.656	0.64
19	Permethylenated 2-Oxabicyclo[4.4.0]dec-9-en-8-one	19.782	1.29
20	1,3,7,7-tetramethyl-, (-)-(1R,3S, 6R)- 2-Methyl-5-cyanohexene	19.891	2.79

Peak No.	Compound Name	R.T.	Area %
21	beta.-D-Glucopyranoside	20.034	0.59
22	tetrahydro geranyl acetate	20.160	1.62
23	3-Cyclohexene-1-ethanol	20.560	0.75
24	Alloocimene	20.709	7.20
25	Ethanone	20.864	7.36
26	Hydroxydihydroedulan	21.081	5.74
27	Octacosane	21.602	1.24
28	1-Heptadecanamine	23.547	1.37
29	Silane, 1,6-heptadiyne-1,7-diylbistrimethyl	24.205	0.68
30	Didodecyl phthalate	24.960	0.77
31	Octane	25.315	0.62
32	Pentatriacontane	25.424	0.89
33	Heneicosanoic acid	25.859	1.73
34	Hexadecane	26.471	0.80
35	Heptadecanoic acid	26.854	0.83
36	1,1- ideuterio-hexadecanyl methane sulfonated.	28.222	0.65
37	Octadecane	28.508	0.68
38	Heneicosane	28.617	0.75
39	Heptadecanoic acid	29.246	1.05
40	1,2-Benzenedicarboxylic acid	32.822	31.15

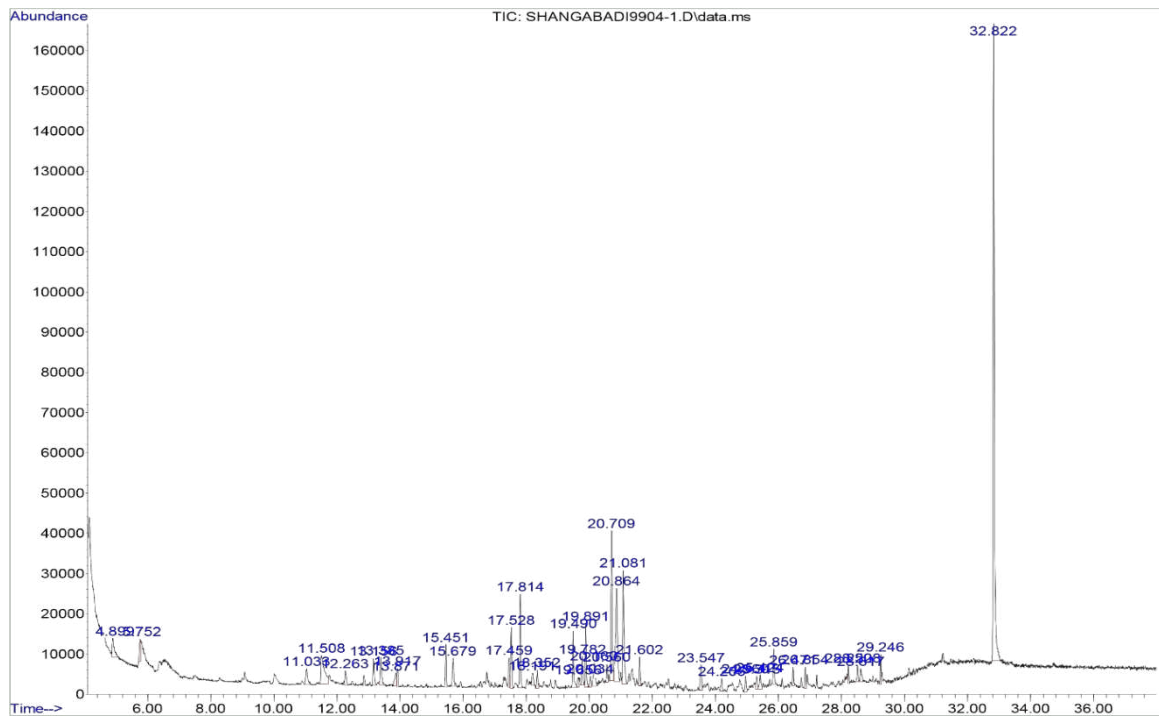


Figure 1. Spectrum of GC-MS Humulus lupulus essential oil constituents in water

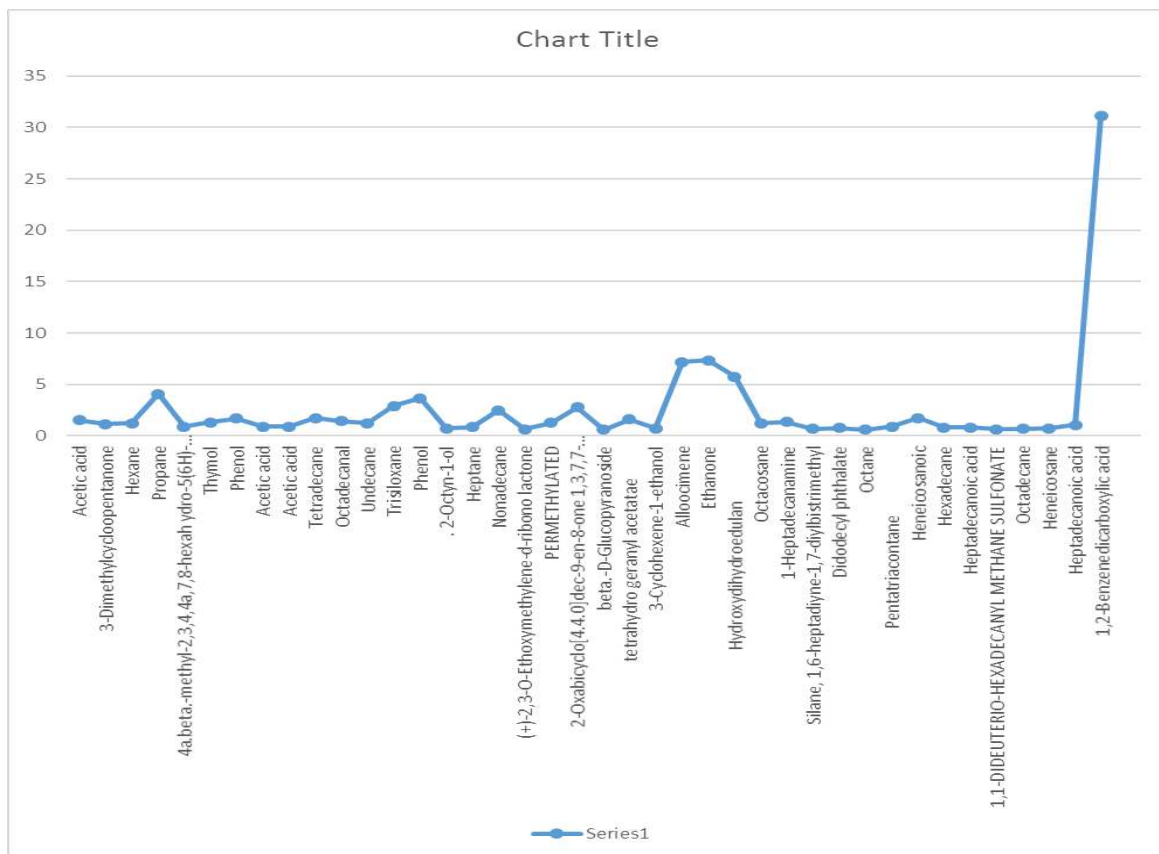


Figure 2. The amount of phenolic compounds in Humulus lupulus in water

Extraction of Malva Sylvestris with water

In the extraction method of Malva sylvestris with water in the ultrasound device, 40 chemical components with different percentage efficiencies were identified. The percentage and type of compounds identified were analyzed by GC-MS. The

chemical compounds observed in each of the medicinal plants were different because the medicinal plants have different chemical compounds in them after extraction and analysis. The following are 40 chemical compounds observed in the presence

of this medicinal plant with different efficiency percentages, which are as follows:

In peak number 1 at RT=3.659 with a purity of 46.23 % is related to Dimethylphosphinic azide. In peak number 2 at RT=5.318 with a purity of 12.89 % is related to Oxime. In peak number 3 at RT= 7.716 with a purity of 7.52 % is related to Phenyl acetaldehyde. In peak number 4 at RT= 8.420 with a purity of 0.32 % is related to Cyclotrisiloxane. In peak number 5 at RT= 9.061 with a purity of 0.51 % is related to Acetamide. In peak number 6 at RT= 10.039 with a purity of 1.53 % is related to decamethyl. In peak number 7 at RT= 11.578 with a purity of 1.68 % is related to Propane. In peak number 8 at RT= 11.773 with a purity of 0.80 % is related to 2-Methyl-5-(4'-methylphenyl)sulfon. In peak number 9 at RT= 11.927 with a purity of 0.43 % is related to 2-Decyne. In peak number 10 at RT= 12.248 with a purity of 0.89 % is related to Acetylcodeine. In peak number 11 at RT= 13.180 with a purity of 0.38 % is related to 1-Vinyl -2,6,6-Trimethyl Cyclohexil-1- ene. In peak number 12 at RT= 13.386 with a purity of 0.41 % is related to 1-Vinyl -2,6,6-Trimethyl Cyclohexil-1- ene. In peak number 13 at RT= 13.924 with a purity of 1.41 % is related to Cyclohexasiloxane. In peak number 14 at RT= 15.132 with a purity of Cyclopentasiloxane 0/6% is related to w . In peak number 15 at RT= 15.446 with a purity of 0.39 % is related to Hydroxylamine. In peak number 16 at RT= 15.601 with a purity of 0.29 % is related to Cholestan-7-ol. In peak number 17 at RT= 16.247 with a purity of 0.27 % is related to 2-cyclohexyl-1,3-dioxolan-4-one. In peak number 18 at RT= 16.974 with a purity of 3.15 % is related to 1-Cyclohexyl-1-pentyne. In peak number 19 at RT= 17.300 with a purity of 0.60 % is related to 4,4-Dimethyl-5-ethylcyclopent-2-en-1-one. In peak number 20 at RT= 17.483 with a purity of 0.64 % is related to (2-Methyl-cyclohex-2-enylidene)-a etaldehydec. In peak number 21 at RT= 17.546 with a purity of 0.98 % is related to Pentasiloxane. In peak number 22 at RT= 17.741 with a purity of 1.09 % is related to 6,6-Dimethylhepta-2,4-diene. In peak number 23 at RT= 18.021 with a purity of 0.33 % is related to 1-Benzoxirene.

In peak number 24 at RT= 18.330 with a purity of 1.23 % is related to 2(4H)-Benzofuranone. In peak number 25 at RT= 18.416 with a purity of 0.99 % is related to (E,E)-.alpha.-farnesene. In peak number 26 at RT= 18.593 with a purity of 0.59 % is related to 5-(2,3-bis(trimethyl silyloxy)propyl)-1,3-dimethyl -5-(1-methyl butyl) barbituric acid. In peak number 27 at RT=19.274 with a purity of 0.45 % is related to 5,8-Dimethylisoquinoline. In peak number 28 at RT= 19.486 with a purity of 0.38 % is related to 1,4-dideuterio-2-methylbutane. In peak number 29 at RT= 20.859 with a purity of 2.56 % is related to 1-Oxaspiro[4.5]decan-2-one. In peak number 30 at RT= 21.260 with a purity of 0.35 % is related to (E)-4-(1'-Acetyl-2',2'-epoxymethan o-5',5'-dimethyl-1'-cyclopentyl)-3 buten-2-one. In peak number 31 at RT= 21.615 with a purity of 0.46 % is related to 5-exo-methyl-5-endo-nitrobicycloheptan-2-one. In peak number 32 at RT= 21.889

with a purity of 0.28 % is related to Hexanoic acid. In peak number 33 at RT= 23.005 with a purity of 0.93 % is related to Hepta-2,4-dienoic acid. In peak number 34 at RT= 23/491 with a purity of 1.88% is related to w . In peak number 35 at RT= 24.207 with a purity of 0.93 % is related to Phenol. In peak number 36 at RT= 24.487 with a purity of 1.38% is related to 1-Monolinoleoylglycerol trimethylsilyl ether. In peak number 37 at RT= 25.712 with a purity of 0.34 % is related to 2-Isopropyl-5-oxohexanal. In peak number 38 at RT= 25.860 with a purity of 2.69 % is related to Hexadecanoic acid. In peak number 39 at RT= 28.149 with a purity of 0.25 % is related to (R)-(-)-14-Methyl-8-hexadecyn-1-ol. In peak number 40 at RT= 28.218 with a purity of 1.48% is related to 9-Octadecenoic acid.

Table 2. Malva sylvestris essential oil constituents in water

Peak No.	Compound Name	R.T.	Area %
1	Dimethylphosphinic azide	3.659	46.23
2	Oxime	5.318	12.89
3	Phenyl acetaldehyde	7.716	7.52
4	Cyclotrisiloxane	8.420	0.32
5	Acetamide	9.061	0.51
6	Cyclopentasiloxane	10.039	1.53
7	Propane	11.578	1.68
8	2-Methyl-5-(4'-methylphenyl)sulfon	11.773	0.80
9	2-Decyne	11.927	0.43
10	Acetylcodeine	12.248	0.89
11	1-vinyl-2,6,6-trimethylcyclohex-1- ene	13.180	0.38
12	1-vinyl-2,6,6-trimethylcyclohex-1- ene	13.386	0.41
13	Cyclohexasiloxane	13.924	1.41
14	Cyclopentasiloxane	15.132	0.60
15	Hydroxylamine.	15.446	0.39
16	Cholestan-7-ol	15.601	0.29
17	2-cyclohexyl-1,3-dioxolan-4-one	16.247	0.27
18	1-Cyclohexyl-1-pentyne	16.974	3.15
19	4,4-Dimethyl-5-ethylcyclopent-2-en-1-one	17.300	0.60
20	(2-Methyl-cyclohex-2-enylidene)-acetaldehyde	17.483	0.64

Peak No.	Compound Name	R.T.	Area %
21	Pentasiloxane	17.546	0.98
22	6,6-Dimethylhepta-2,4-diene	17.741	1.09
23	1-Benzoxirene	18.021	0.33
24	2(4H)-Benzofuranone	18.330	1.23
25	(E,E)-.alpha.-farnesene	18.416	0.99

26	5-(2,3-bis(trimethyl silyloxy)propyl)-1,3-dimethyl-5-(1-methyl butyl) barbituric acid	18.593	0.59	32	Hexanoic acid	21.889	0.28
27	5,8-Dimethylisoquinoline	19.274	0.45	33	Hepta-2,4-dienoic acid	23.005	0.93
28	1,4-dideuterio-2-methylbutane	19.486	0.38	34	Phenol	23.491	1.88
29	1-Oxaspirodecan-2-one	20.859	2.56	35	1-Monolinoleoylglycerol trimethylsilyl ether	24.207	0.39
30	(E)-4-(1'-Acetyl-2',2'-epoxymethan	21.260	0.35	36	2-Isopropyl-5-oxohexanal	24.487	1.38
31	5-exo-methyl-5-endo-nitrobicyclo heptan-2-one	21.615	0.46	37	Undecanal	25.712	0.34
				38	Hexadecanoic acid	25.860	2.69
				39	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	28.149	0.25
				40	9-Octadecenoic acid	28.218	1.48

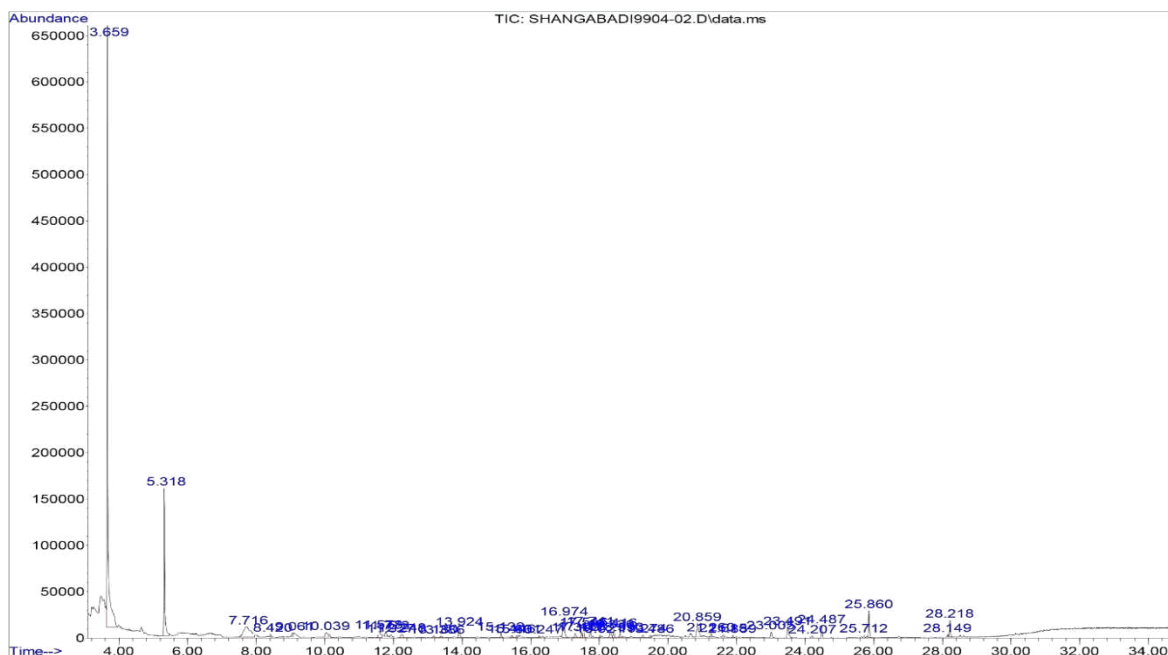


Figure 3. Spectrum of GC-MS Malva Sylvestris essential oil constituents in water

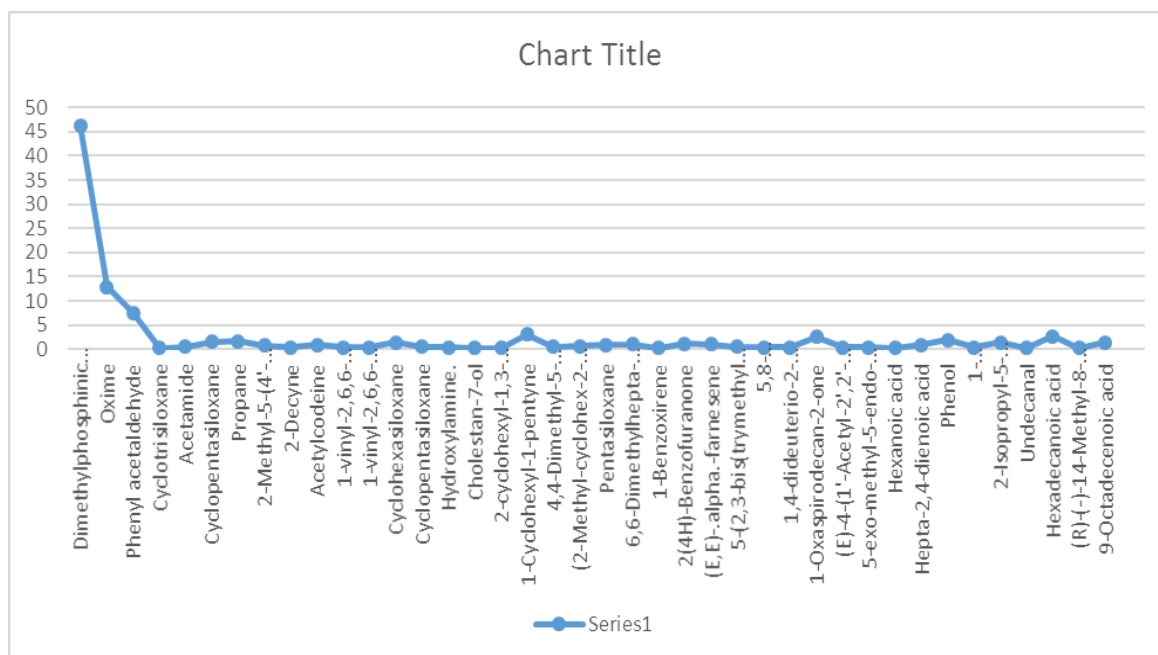


Figure 4. The amount of phenolic compounds in Malva sylvestris in water

Extraction of thymus with water

In the extraction method of Thymus with water in the ultrasound device, 40 chemical components with different percentage efficiencies were identified. The percentage and type of compounds identified were analyzed by GC-MS. The chemical compounds observed in each of the medicinal plants were different because the medicinal plants have different chemical compounds in them after extraction and analysis. The following are 40 chemical compounds observed in the presence of this medicinal plant with different efficiency percentages, which are as follows:

In peak number 1 at RT=7.619 with a purity of 5.81 % is related to Benzeneacetaldehyde. In peak number 2 at RT=10.869 with a purity of 0.94 % is related to 1,5-Octadiene-3,7-diol, 3,7-dimethyl-(terpenediol I). In peak number 3 at RT=11.853 with a purity of 1.01% is related to Methylenelethylmaleimid. In peak number 4 at RT=12.259 with a purity of 0.74 % is related to 6H-Dibenzo[a,g]quinolizin-1-ol,. In peak number 5 at RT=13.146 with a purity of 5.26 % is related to Phenol. In peak number 6 at RT=13.266 with a purity of 0.71 % is related to Benzeneacetonitrile. In peak number 7 at RT=13.375 with a purity of 7.67 % is related to Pheno. In peak number 8 at RT=13.724 with a purity of 0.77% is related to Bicyclohex-3-en-2-one, 4-methyl-1-(1-methylethyl)-u mbellulone. In peak number 9 at RT=16.865 with a purity of 1.64 % is related to (Z)-Cis-.Alpha.-bergamotene. In peak number 10 at RT=16.968 with a purity of 4.47 % is related to 4-Methylphenoxyacetonitrile. In peak number 11 at RT=17.300 with a purity of 3.09 % is related to 4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one. In peak number 12 at RT=17.746 with a purity of 0.97 % is related to 1,5-dimethylbicyclooctane. In peak number 13 at RT=18.324 with a purity of 1.72 % is related to 2(4H)-Benzofuranone. In peak number 14 at RT=18.416 with a purity of 2.17 % is related to 2,7-dimethyl-5-methyliden -tricyclodec-7-en-4-ol. In peak number 15 at RT=18.582 with a purity of 0.93 % is related to 3. In peak number 16 at RT=18.897 with a purity of 0.80 % is related to Megastigmatrienone. In peak number 17 at RT=19.234 with a purity of 3.06 % is related to Megastigmatrienone. In peak number 18 at RT=19.721 with a purity of 10.53 % is related to Methyl .beta.-d-galactopyranoside . In peak number 19 at RT=19.898 with a purity of 1.55 % is related to alpha.-D-Glucopyranoside. In peak number 20 at RT=20.133 with a purity of 2.32 % is related to Megastigma trienone. In peak number 21 at RT=21.254 with a purity of 0.72 % is related to 3-Buten-2-one, 4-(2,2,6-trimethyl- Phenol, 3-amino-4-methyp-Cresol, 3-amino-2-Amino-4-hydroxymethylphenol. In peak number 22 at RT=21.603 with a purity of 1.53 % is related to Bicycloheptane, 2,2,3-trimethyl-, endo- Norbornane. In peak number 23 at RT=22.376 with a purity of 0.71 % is related to 5-Ethylcyclopent. In peak number 24 at RT=22.690 with a purity of 0.92 % is related to Tetradecanoic acid. In peak number 25 at RT=22.913 with a purity of 1.96 % is related to Benzyl

benzoate. In peak number 26 at RT=23.005 with a purity of 4.12 % is related to (-)-Loliolide. In peak number 27 at RT=23.491 with a purity of 1.74 % is related to Benzene. In peak number 28 at RT=24.361 with a purity of 0.90 % is related to Diallyldivinylsilane. In peak number 29 at RT=24.487 with a purity of 3.37 % is related to 2-Pentadecanone. In peak number 30 at RT=25.528 with a purity of 4.17 % is related to 9-Hexadecenoic acid.

In peak number 31 at RT=25.797 with a purity of 1.23 % is related to Cyclohexanone. In peak number 32 at RT=25.860 with a purity of 4.25 % is related to Hexadecanoic acid. In peak number 33 at RT=26.427 with a purity of 8.02 % is related to Hexadecanoic acid. In peak number 34 at RT=28.218 with a purity of 2.58 % is related to 9-Octadecenoic acid. In peak number 35 at RT=28.281 with a purity of 2.65 % is related to 9-Octadecenoic acid. In peak number 36 at RT=28.366 with a purity of 0.81 % is related to Phytol. In peak number 37 at RT=28.509 with a purity of 1.46 % is related to Octadecanoic acid. In peak number 38 at RT=29.934 with a purity of 0.98 % is related to -11,13-Dimethyl-12-tetradecen-1-o. In peak number 39 at RT=30.861 with a purity of 0.90 % is related to 4,8,12,16-Tetramethylheptadecan-4-. In peak number 40 at RT=32.824 with a purity of 0.80 % is related to Bis(2-ethylhexyl) phthalate.

Table 3. Thymus essential oil constituents in water

Peak No.	Compound Name	R.T.	Area %
1	Benzeneacetaldehyde	7.619	5.81
2	1,5-Octadiene-3,7-diol	10.869	0.94
3	1H-Pyrrole-2,5-dione	11.853	1.01
4	6H-Dibenzoquinolizin-1-ol	12.259	0.74
5	Phenol	13.146	5.26
6	Benzeneacetonitrile	13.266	0.71
7	Phenol	13.375	7.67
8	Bicyclo[3.1.0]hex-3-en-2-one, 4-methyl-1-(1-methylethyl)	13.724	0.77
9	(Z)-Cis-.Alpha.-bergamotene	16.865	1.64
10	4-Methylphenoxyacetonitrile	16.968	4.47
11	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one	17.300	3.09
12	1,5-dimethylbicyclo octane	17.746	0.97
13	2(4H)-Benzofuranone	18.324	1.72
14	2,7-dimethyl-5-methyliden -tricyclodec-7-en-4-ol	18.416	2.17
15	1H-Pyrrole	18.582	0.93
16	Megastigmatrienone	18.897	0.80
17	Megastigmatrienone	19.234	3.06
18	Methyl .beta.-d-galactopyranoside	19.721	10.53
19	alpha.-D-Glucopyranoside	19.898	1.55
20	Megastigma trienone	20.133	2.32

Peak No.	Compound Name	R.T.	Area %
21	3-Buten-2-one	21.254	0.72
22	Bicycloheptane, 2,2,3-trimethyl-, endo	21.603	1.53
23	5-Ethylcyclopent-1-ene-1-carboxylic acid	22.376	0.71
24	Tetradecanoic acid	22.690	0.92
25	Benzyl benzoate	22.913	1.96
26	(-)-Loliolide	23.005	4.12
27	Benzene	23.491	1.74
28	Diallyldivinylsilane	24.361	0.90
29	2-Pentadecanone	24.487	3.37
30	9-Hexadecenoic acid	25.528	4.17
31	Cyclohexanone	25.797	1.23
32	Hexadecanoic acid	25.860	4.25
33	Hexadecanoic acid	26.427	8.02
34	9-Octadecenoic acid	28.218	2.58
35	9-Octadecenoic acid	28.281	2.65
36	Phytol	28.366	0.81
37	Octadecanoic acid	28.509	1.46
38	E-11,13-Dimethyl-12-tetradecen-1-ol acetate	29.934	0.98
39	4,8,12,16-Tetramethylheptadecan-4-olide	30.861	0.90
40	Bis(2-ethylhexyl) phthalate	32.824	0.80

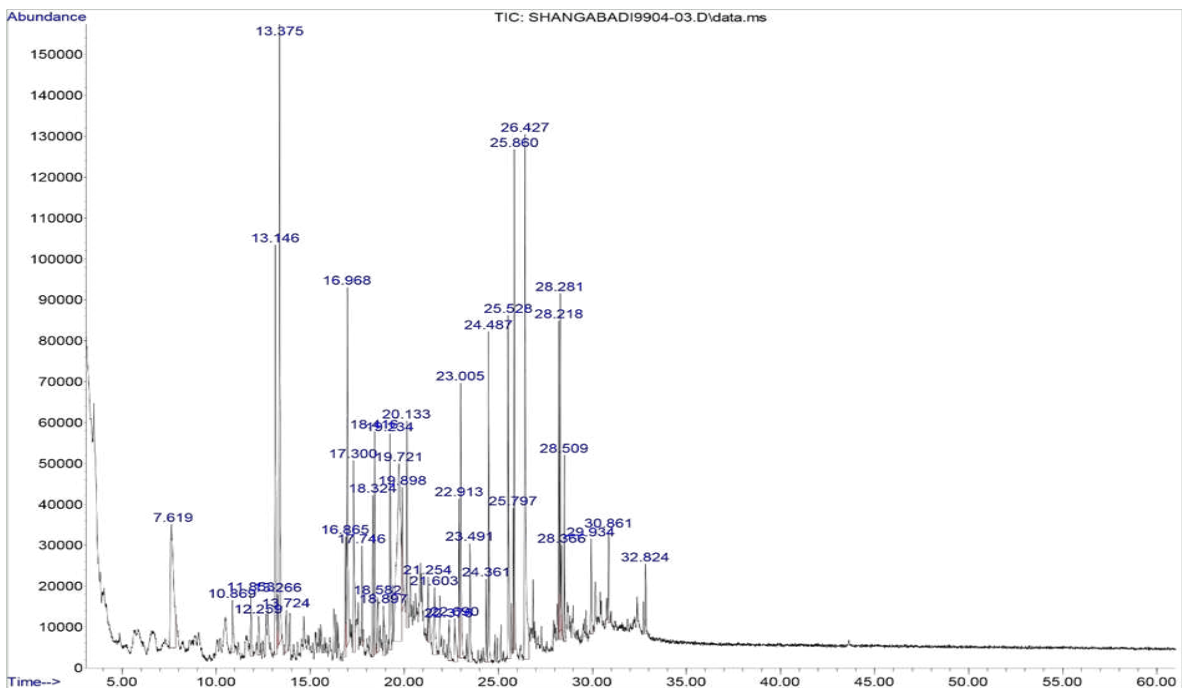


Figure 5. Spectrum of GC-MS Thymus essential oil constituents in water

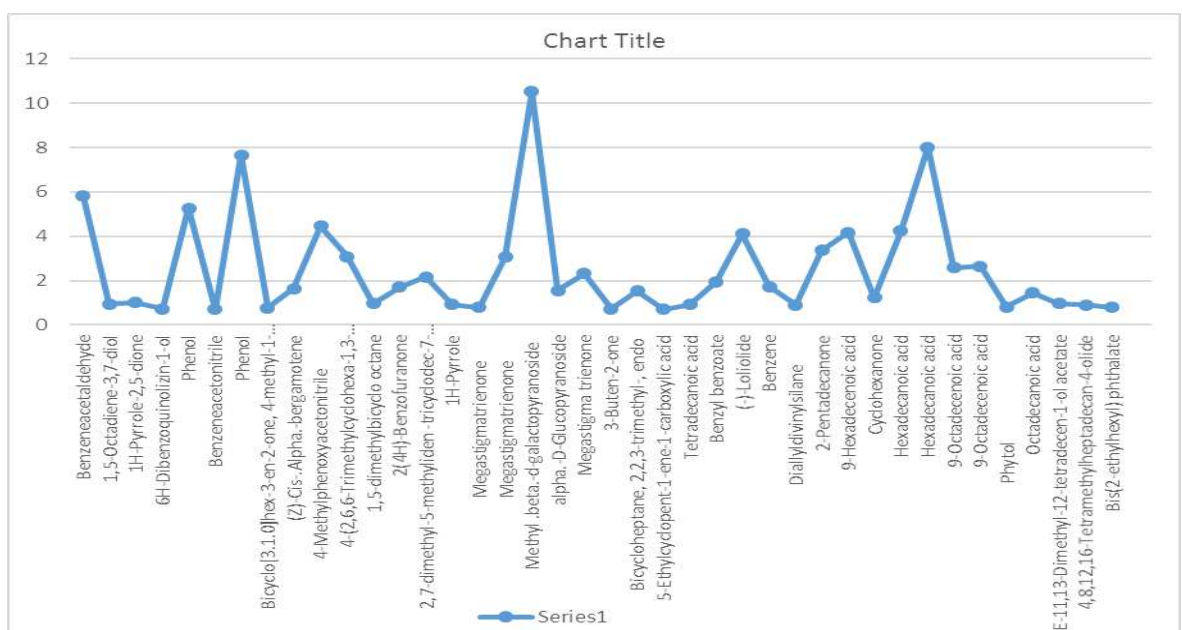


Figure 6. The amount of phenolic compounds in Thymus in water

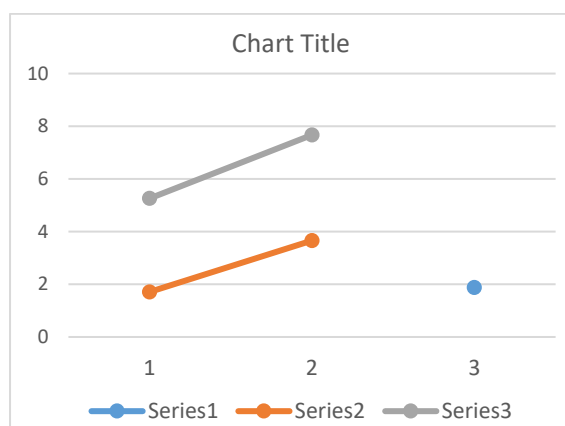


Figure 7. Comparison the phenolic compounds in three plants of *Humulus lupulus*, *Malva sylvestris* and *Thymus* in the three different extraction solvents mentioned above, the orange line of *Humulus lupulus* in water, the dark line of *Malva sylvestris* in water and the orange dot of *thymus* in water.

Discussion and Results

In analysis with *Humulus lupulus*, 40 peaks were identified in this compound, the highest percentage efficiency with 31.15% related 1,2-Benzenedicarboxylic acid and the lowest percentage efficiency with 0.59% related to beta.-D-Glucopyranoside.

In analysis with *Malva Sylvestris*, 40 peaks were identified in this compound, the highest percentage efficiency with 46.26% related to Dimethylphosphinic azide and the lowest with 0.25% related to (R)-(-)-14-Methyl-8-hexadecyn-1-ol.

In analysis with *Thymus*, 40 peaks were identified in this combination, with the highest percentage efficiency with 10.53% related to Methyl .beta.-d-galactopyranoside and the lowest percentage efficiency with 0.73% related to 5-Ethylcyclopent-1-ene-1-carboxylic acid. Therefore, the combination of phosphinic acid with the highest percentage efficiency is recommended as a drug combination in therapeutic fields, which has more antioxidant properties and water solvent was introduced as the most polar solvent.

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