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Using GC-MS Technology to Identify the Compounds Resulting from Mixing of Alcoholic Extracts of Some Medicinal Plants

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ABSTRACT

Phytochemicals are considered as one of the most important pharmaceutical compounds used in pharmacy at all, due to their high therapeutic efficacy and few side effects compared with chemically prepared drugs. On the other hand, the modern techniques of analysis such as GC-MS made it easier to know the chemicals that make up the materials to be studied. In our study, alcoholic extracts of (*Punica granatum* peel, *Ziziphusspina-christi* leaves, and *Eucalyptus camaldulensis* leaves) were prepared, in addition to prepare the mixtures of these extracts by mixing the equal proportions of two extracts only of three plant extracts each time. The result was 3 pure extracts and 3 mixture extracts. After that, these samples were analyzed with the GC-MS device, and the results of the analysis were compared with each other to find out the structural differences occurred after mixing. The results proved the presence of new chemical compounds in the mixture extracts completely different from the chemical compounds found in the pure compounds, whether in terms of chemical composition or biological activity.

GRAPHICAL ABSTRACT

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Introduction

Pure compounds from natural sources or pure plant extracts can be considered as sources for newer drugs [1]. World Health Organization (WHO) considered that about 80% of humans rely on folk medicine for their primary healthcare care. The use of medicines from herbal sources in Asia gives a long history of contact between human and the environment. In traditional medicine, there are phytochemicals used to treat chronic diseases and infections [2]. Because of the side effects and bacterial resistance in synthesized drugs, human turned to use botany. There are thousands of chemical compounds from plants sources that are safe and effective alternatives with fewer side effects [3].

Punica granatum; a granular apple [4]. Pomegranate from the family *punicaceae* are fruits to the Mediterranean region. The edible part of the fruit contains many phytochemicalsas saccharides, polyphenol, and some minerals. The major class of pomegranate chemicals is the polyphenols such as phenolic rings with many hydroxyl groups. Pomegranate polyphenols comprise flavonoids such as flavanols, flavonols and anthocyanins, also condensed tannins and hydrolysable tannins [5].

Eucalyptus camaldulensis is an important plant belonging to the family, *Myrtaceae*, is used as a medicine for treatment of sore throat and some other bacterial infection of the respiratory and urinary tracts. The essential oils in the leaves are used as an expectorant to treat lung disease [6]. The alcoholic extract and the aqueous extract of *Eucalyptus camaldulensis* were found to be effective in inhibiting different types of Gram-positive and Gram-negative bacteria [7].

Ziziphusspina-christi has important fruits that usually eaten freshly. The fruits can be applied on wounds and ulcers [8, 9]. Pneumonia, dysentery, scorpion bites, cough, constipation, intestinal worms, and fever are some of the uses of this plant [9, 10]. It has shown that extracts of leaves of this plant has anti-microbial, anti-nociceptive, and anti-diabetic effects [11]. The extract of *Ziziphusspina-christi* also contains beutic acid and ceanothic acid, cyclopeptides also contain saponin glycosides, protein, free sugar and mucilage that have the anti-bacterial activity [8 10].

Gas chromatography-mass spectrometry (GC-MS) is considered as a method that performed coupling between the features of gas-chromatography and mass spectrometry to identify different compounds in the sample. GC can separate essential and semi-essential compounds with a great resolution, but it cannot diagnose them. MS can give the whole structural information about most chemicals that can be precisely identified, but it cannot readily isolate them [12].

The study aims to identify the chemical compounds resulting from mixing the following plant extracts (*Punica granatum* peels and *Ziziphusspina-christi*), (*Punica granatum* peels and *Eucalyptus camaldulensis* leaves) and (*Ziziphusspina-christi* and *Eucalyptus camaldulensis* leaves) and compare them with the chemical compounds of these extracts, while they are in their pure state, by using the GC-MS technique, and studying the biological activity of these compounds in theory, to make room for many other studies carried out by mixing plant extracts, identifying the resulting compounds, and studying the chemical reactions that occur as a result of the mixing process that led to the production of contrasting compounds, and also an applied study of the effectiveness of these compounds.

Materials and Methods

Collection of the plant material

Punica granatum peel, *Ziziphusspina-christi,* and *Eucalyptus camaldulensis* leaves were collected fresh and free from any abnormal condition or disease. They were collected from local farms in the Holy Karbala/Iraq. The raw materials were washed well with tap water, and then with distilled water more than once. After that, they were dried in the open air for a period ranging between 8-10 days. Next, it is ground by using an electric grinder for a period ranging from 4-6 minutes, and then it was stored in closed bottles until use.

Preparation of alcoholic extracts

Eucalyptus camaldolehsis leaves and *Ziziphusspina-christi* leaves.

20 g of the sample were weighted and put in the Soxhlet with 400 mL ethanol (70% for *Eucalyptus* and 95% for *Ziziphusspina-christi* concentration) for one day [13] (Table 1).

Table 1: Chemical compounds in *Ziziphusspina-christi* leaves, *Punica granatum* peel, *Eucalyptus camaldolehsis* leaves

No.	*Ziziphusspina-christi* leaves	*Punica granatum* peel	*Eucalyptus camaldolehsis* leaves
1	1-Butanol , 3-methyl- , acetate	1-Butanol , 3-methyl- , acetate	1-Butanol , 3-methyl- , acetate
2	1,3-Bis(hydroxymethyl)urea	dl-Alanyl-dl-valine	Acetic acid, [(aminocarbonyl)amino]oxo-
3	Propane, 1-(1-methylethoxy)-	Xanthine, 1,3-dipropyl-8-[(4-carboxymethoxy)phenyl]-	Ethanamine, 2,2'-oxybis-
4	Butane, 1,1-diethoxy-3-methyl-	Acetonitrile,2-[(1-ethyl-1H-1,2,3,4-tetrazol-5-yl) thio]-	Benzene, (2-methoxyethyl)-
5	Ethane, 1,1,1-triethoxy-	(3- Methyl-oxiran-2-yl)-methanol	Propanedioic acid, dihydroxy-
6	Acetic acid, [(benzoylamino)oxy]-	2-Formylhistamine	Propane, 1-(1-methylethoxy)-
7	Hexanoicacid, ethyl ester	3,3-Dimethyl-4-methylamino-butan-2-one	Butane, 1,1-diethoxy-3-methyl
8	1,7,7-Trimethyl-2-vinylbicyclo[2.2.1]hept-2-ene	Dimethylamine	Ethane, 1,1,1-triethoxy-
9	Diethyl Phthalate	Ethanol, 2-(vinyloxy)-	Eucalyptol
10	1-Butanol, 4-mercapto-	2-(4,5-Dihydro- 3-methyl-5-oxo-1-phenyl-4-pyrazolyl)- 5-nitrobenzoic acid	-)-Spathulenol(
11	13-Octadecenal	(S)-(+)-1-Cyclohexylethylamine	Diethyl Phthalate
12	N-[3-[N-Aziridyl]propyl]cyclohexylamine	Formic acid, 1-methylpropyl ester	1, 2, 4- Triazino[5,6-E] [1,2,4]-triazine-3,6 -dione,hexahydro-
13	6-Hydroxy-9-oxa-bicyclo[3.3.1]nonan-3-one	Butane, 1,1-diethoxy-3-methyl-	Hydroxycyprazine
14	1,2 -Benzenedicarboxylic acid, ditridecyl ester	Acetic acid, [(aminocarbonyl)amino]oxo-	Di(pent-4-enyl)amine
15	2-Propyl-5-oxohexanoic acid	Ethyl 2,2 -diethoxypropionate	Fumaric acid, hexadecylpropargyl ester
16	2- Bromopropionic acid, hexadecyl ester	Hexanoicacid, ethyl ester	n-Hexadecanoic acid
17	cis-3 -Methyl- exo-tricyclo [5.2.1.0 (2.6)]decane	1,7,7-Trimethyl -2-vinylbicyclo [2.2.1] hept-2 -ene	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate
18	Hexadecane, 1,1-bis(dodecyloxy)-	Benzoic acid, 4-(1-azepinyl)azo-, ethyl ester	Cyclopentane-trans-1,3-dicarboxamide
19	9- Oxa- bicyclo[3.3.1] nonane -2,6-dione	Phthalic acid, allyl ethyl ester	Trieicosane, 1-bromo-11-docosenyliden-
20	3- Methyl-2- (3-methylpentyl) -3-buten- 1-ol	2,3-Dihydroindole-4-ol-2-one	1,4-Naphthalenediol, decahydro-, (1.alpha.,4.alpha.,4a.alpha.,8a.alpha.)-
21	1,5,9-Cyclododecanetriol	Diethyl Phthalate	Triallylphosphine $$ Phosphine, tri-2-propenyl-
22	Cyclohexanone, 2-(1-hydroxyethyl)-	1,3-Dioxolane, 2-phenyl-2-(phenylmethyl)-	Chloroacetic acid, 2,2-dimethylpropyl ester
23	Quinoline-5,8-dione-6-ol, 7-[[(4-cyclohexylbutyl)amino]methyl]-	Phthalic acid, isoporpyl propyl ester	3-Hexanol, 4,4-dimethyl-
24	Tetracosane, 1-bromo-	Benzofuran-2-one, 4-amino-2,3-dihydro-	Cyclohexanol, 2,2,6,6-tetramethyl-

25	Butanoic acid, 5-hexenyl ester	Phthalic acid, 2-acethylphenyl ethyl ester	Cyclododecanemethanol
26	Tridecanal	Ethyl tridecanoate	Methanesulfonic acid, 2,7-dioxatricyclo [4.3.1.0 (3,8)] dec-5 -yl ester
27	Z,Z -8,10 -Hexadecadien- 1-ol	Tridecanoic acid	Cyclohexane-1,3-dicarboxamide
28	Toluene -4- sulfonic acid, 2,7-dioxatricyclo [4.3.1.0 (3,8)] dec-10-yl ester	1,4-Butanediamine, N-(3-aminopropyl)-	Propanal,3-hexylimino-2-nitro-
29	Dispiro(2,4-dineopentyl-1,3-disilacyclobutan1,2';3,2"-bis[3-neopentyl-2-silatetracyclo[7.0.0 (1,5).0(4,8).0 (7,9)]nonan	1,2 -Benzenedicarboxylic acid,bis(2-methylpropyl)ester	2-Hydroxymethyl-2-methylcyclopentanol
30	Cyclohexanol, 2-methylene-3-(1-methylethyl)-, cis-	n-Hexadecanoic acid	2,2,4 -Trimethyl- 3 -(3,8,12,16-tetramethyl- heptadeca-3,7,11,15 -tetraenyl) cyclohexanol
31	Cyclohexanone, 2,3-dimethyl-2-(3-oxobutyl)-	Ethyl tridecanoate	2-Hydroxypentadecyl propanoate
32	Cyclopropane,1-(1,2 -dimethylpropyl)-1-methyl-2-nonyl-	7-Tetradecene	2H -Azepin-2 -one, 5-(1,1-dimethylethyl) hexahydro-
33	1-Pentatriacontanol	2-Trifluoroacetoxydodecane	Piperidine, 3,5-dimethyl-
34	Trichloroacetic acid, undecyl ester	3-Hexadecyne	7-Tetradecenal, (Z)-
35	Cyclohexane, (3,3-dimethylpentyl)-	7-Tetradecenal, (Z)-	Bicyclo[2.2.1]heptan-7-one-1-carboxylic acid, ethylene ketal-, t-butyl ester
36	Octanoic acid, 2-butyl ester	Acetic acid,1,3,7-trimethylocta- 2,6-dienyl ester	Bicyclo[2.2.1]heptan-2-one, 5-hydroxy-4,7,7-trimethyl-, endo-
37	1,3-Cyclohexanediol	Exo- tricycle [6.2.1.0(2.7)] undecane	Cyclohexanone, 2,3-dimethyl-2-(3-oxobutyl)-
38	beta.-D-Glucopyranoside, 4-nitrophenyl	8,9,9,10,10,11 -Hexafluoro- 4,4-dimethyl -3,5-dioxatetracyclo [5.4.1.0(2,6).0(8,11)]dodecane	2-Carboxymethyl-3-methyl-cyclopentanecarboxylic acid
39	1,6-Cyclodecanediol	Octadecanoic acid	1-Heptadec-1-ynyl-cyclohexanol
40	Propanal,3-hexylimino-2-nitro-	Cyclohexanecarboxylic acid, 2-ethylcyclohexyl ester	Octadecanoic acid

**The cells marked with the same color indicate the common chemical compounds appeared in the pure extracts*

Punica granatum Peel

20 g of sample with 400 mL of ethanol (70%) was mixed by magnetic stirrer for 2 hours, and then the sample was steeped for one day, filtered, and centrifuged (3000 C/min for 10 minutes) [14].

Preparation of mixture extracts

3 mixture extracts were prepared by mixing 30 mL of each extract of the pure extracts and mixing it with 30 mL of the other extract by means of a magnetic stirrer for 3 minutes after which the resulting mixture extracts were stored in refrigeration until use.

Analysis by using GC-MS technology

30 mL of each sample of the extracts (pure and mixed) were taken and sent well cooled to the GC MS lab in the Department of Food Sciences at the College of Agriculture/University of Basra/Iraq (Table 2).

Table 2: Analysis of GC-MS for mixtures alcoholic extracts

Peak	GC-MS. For Mix. *of Punica granatum peel & Ziziphusspina-christi leaves*
1	1-Butanol, 3-methyl-, acetate $$ Isoamylethanoate
5	Propane, 1-(1 -methylethoxy)- $$ Ether,isopropyl propyl
6	Ethanol, 2-(vinyloxy)- $$ Ethylene glycol monovinyl ether $$ Ethanol, 2-(ethenyloxy)- $$ Ethylene glycol vinyl ether $$ Ethyleneglycolmonovinyl ester $$ Vinyloxyethanol
7	2-Isopropoxyethylamine $$ 2-Isopropoxyethanamine
13	1,1-Diethoxy-3-methylbutane
14	Ethyl 2,2-diethoxypropionate $$ Ethyl 2,2-diethoxypropanoate
16	Benzene, 1,2,4 -trimethyl - $$ .psi.-Cumene
17	Hexanoic acid , ethyl ester $$ Ethyl caproate
19	Acetic acid, cyano- $$ Cyanoacetic acid $$ Malonicmononitrile $$ Monocyanoacetic acid $$ NCCH2COOH $$ 2-Cyanoacetic acid $$ Acidecyanacetique $$ USAF kf-17
21	3-Isothiazolecarboxamide, 4-amino-
22	1-Adamantanemethylamine, .alpha.-methyl- $$ .alpha.-Methyladamantanemethylamine $$ .alpha.-Methyl-1-adamantanemethylamine $$ Rimantadine
24	N-(4-[(4-Cyano-3,7-dimethylpyrido[1,2-a]benzimidazol-1-yl)amino]phenyl)acetamide
27	Propanedioic acid, dihydroxy- $$ 2,2-Dihydroxymalonic acid
28	Methoxyacetic acid, octyl ester $$ Octylmethoxyacetate
30	Benzene, (1,1,2-trimethylpropyl)- $$ 2,3-Dimethyl-2-phenylbutane $$ (1,1,2-Trimethylpropyl)benzene
32	Acetic acid, oxo - $$ Glyoxylic acid
33	Benzene, m -diisopropyl
35	Diethyl Phthalate
36	2-Propyl-tetrahydropyran-3-ol
37	n-Hexadecanoic acid
38	4-Chloro-3-n-hexyltetrahydropyran $$ 4-Chloro-3-hexyltetrahydro-2H-pyran
Peak	GC-MS. for mix of *Ziziphusspina-christi* leaves and Eucalyptus camaldulensis leaves
1	1-Butanol, 3-methyl-, acetate $$ Isoamylethanoate acetate
3	Propanedioic acid, dihydroxy- $$ 2,2-Dihydroxymalonic acid
7	Bicyclo [2.1.1]hex-2 -ene, 2-ethenyl-
8	Propane, 1-(1-methylethoxy)-
10	Butane, 1,1-diethoxy-3-methyl-
11	Ethane, 1,1,1-triethoxy- $$ Orthoacetic acid, triethyl ester $$ Ethyl orthoacetate $$ Triethylorthoacetate $$ 1,1,1-Triethoxyethane
12	Benzene, 1-ethyl-3-methyl- $$ Toluene, m-ethyl-
13	Hexanoic acid,ethyl ester
14	Eucalyptol $$ Cineole $$ 2-Oxabicyclo[2.2.2]octane, 1,3,3 -trimethyl
15	Methoxyacetic acid, 2-pentyl ester
16	4-Amino-2-oxy-furazan-3-carboxylic acid
18	Acetic acid, ethoxyhydroxy-, ethyl ester $$ Ethyl ethoxy(hydroxy)acetate

19	(3-Methyl-oxiran-2-yl)-methanol
20	1,3-Cyclopentadiene, 1,2,3,4-tetramethyl-5-methylene-
21	7,10-Epoxytricyclo[4.2.1.1(2,5)]decane, 1-trimethylsilyl-
23	Benzene, 1,4-bis(1-methylethyl)-
24	Acetic acid, oxo- $$ Glyoxylic acid
25	2-(4,5-Dihydro-3 -methyl-5-oxo -1-phenyl-4-pyrazolyl)-5 -nitrobenzoic acid
26	(-)-Spathulenol
27	Diethyl Phthalate
31	1,4 –Dioxaspiro [4.6] undec-8-ene, 7,10-methano-
33	n-Hexadecanoic acid $$ Hexadecanoic acid
38	Cyclopentanecarboxylic acid,
Peak	GC-MS for mix of *Eucalyptus camaldulensis* leaves and *Pomegranate* peel*s*
1	1-Butanol, 3-methyl-, acetate $$ Isoamylethanoate
2	(S)-(+)-1-Cyclohexylethylamine $$ 1-Cyclohexylethanamine
3	(S)-Isopropyl lactate $$ Propanoic acid, 2-hydroxy-, 1-methylethyl ester, (S)- $$ Isopropyl 2-hydroxypropanoate
5	Propane, 1- (1-methylethoxy) -
9	Butanoic acid, 3-hydroxy- $$ Butyric acid, 3-hydroxy-
11	(2S,4S)-(+)-Pentanediol $$ 2,4-Pentanediol
12	Propanedioic acid, dihydroxy- $$ 2,2-Dihydroxymalonic acid
13	Butane, 1,1-diethoxy-3-methyl-
14	Ethane, 1,1,1-triethoxy- $$ Orthoacetic acid, triethyl ester $$ Ethyl orthoacetate $$ Triethylorthoacetate $$ 1,1,1-Triethoxyethane
16	Ketone, methyl 4-pyridyl, O-acetyloxime $$ (1E)-1-(4-Pyridinyl)ethanone o-acetyloxime
17	Hexanoic acid, ethyl ester $$ Ethyl caproate
18	Propanedioic acid, dihydroxy- $$ 2,2-Dihydroxymalonic acid
21	Benzene, 1-methyl-4-(1-methylethyl)- $$ p-Cymene
22	Eucalyptol
23	Diglycerol $$ 1,2-Propanediol, 3,3'-oxybis- $$ .alpha.,.alpha.'-Diglycerol $$ Diglycerine
24	1,3,5-Cycloheptatriene, 3,7,7-trimethyl- $$ 3,7,7-Trimethyl-1,3,5-cycloheptatriene
25	2-(4,5-Dihydro-3 -methyl-5-oxo-1 -phenyl-4 -pyrazolyl)-5-nitrobenzoic
28	1,7,7-Trimethyl-2-vinylbicyclo[2.2.1]hept-2-ene
30	Cyclopentanemethanol, .alpha.-methyl- $$ 1-Cyclopentylethanol
31	Cyclohexanepropanol, .alpha.-methyl- $$ 4-Cyclohexyl-2-butanol
33	trans-Z-.alpha.-Bisabolene epoxide
34	Diethyl Phthalate
37	Clonitazene
38	n -Hexadecanoic acid
39	2,4-Decadien-1-ol $$ (2E,4E)-2,4-Decadien-1-ol
40	Threitol,2-O -octyl-

Results and Discussion

Alcoholic plant extracts and their mixtures

Figure 1 shows the types of plant alcoholic extracts of *Ziziphus spina - christi* leaves, *Eucalyptus camaldolehsis* leaves, and *Punica granatum* Peel.

Figure 2 displays the mixture alcoholic extracts of (*Ziziphus spina - christi Eucalyptus camaldolehsis), (Ziziphus spina-Christi and Punica granatum)*, and *(Eucalyptus camaldolehsis and Punica granatum).*

Analysis of GC-MS for pure alcoholic plant extracts

Figure 3 depictes chromatogram of *Eucalyptus camaldolehsis* leaves extracts by using GC-MS technology.

Figure 1: Plants extracts

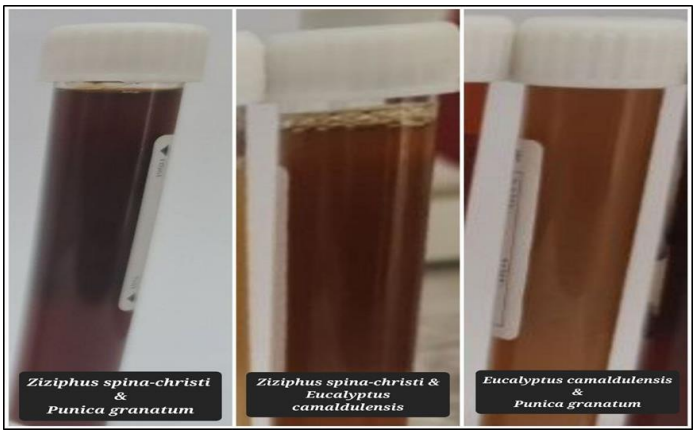

Figure 2: The mixture alcoholic extracts of *Ziziphus spina - christi Eucalyptus camaldolehsis, Ziziphus spina – Christi and Punica granatum*, and *Eucalyptus camaldolehsis and Punica granatum*

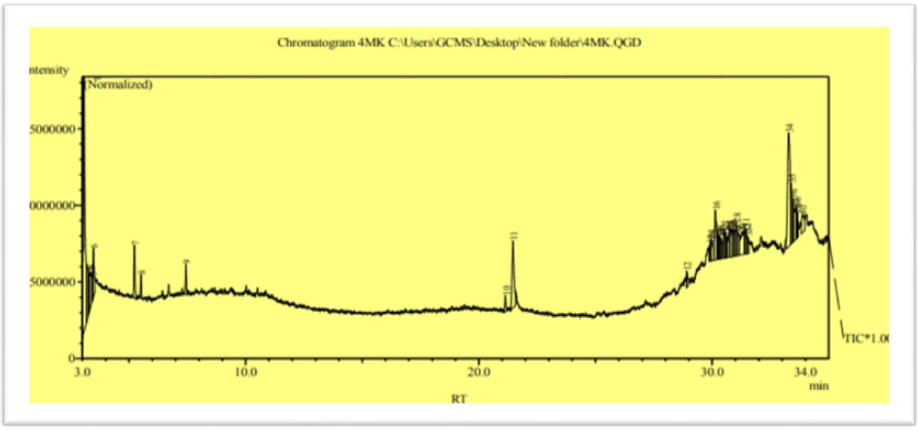

Figure 3: Chromatogram of *Eucalyptus camaldolehsis* leaves extracts by using GC-MS technology

Figures 4 displays chromatogram of *Ziziphus spina-christi* leaves extracts by using GC-MS technology.

Figures 5 illustrates chromatogram of *Punica granatum* peel extract by using GC-MS technology.

Analysis of GC-MS for mixture extracts

Figures 6 demonstrates chromatogram of mixture extracts (*Ziziphus spina - christi* leaves and *Eucalyptus camaldolehsis* leaves) by using GC-MS technology.

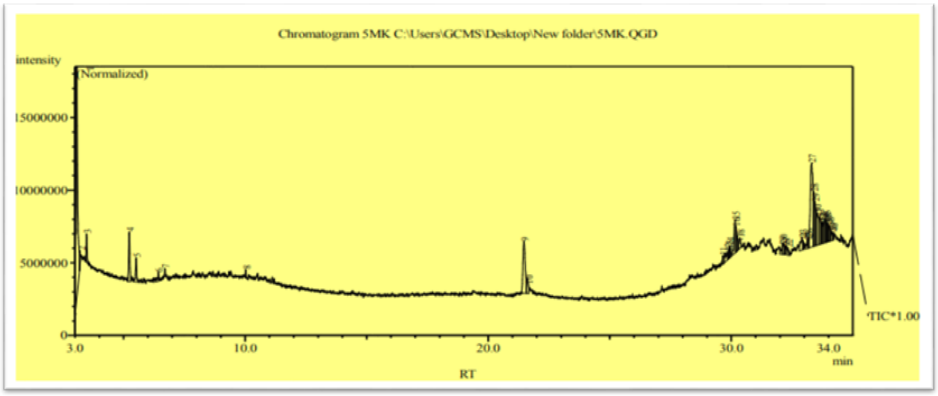

Figure 4: Chromatogram of *Ziziphus spina - christi* leaves extracts by using GC-MS technology

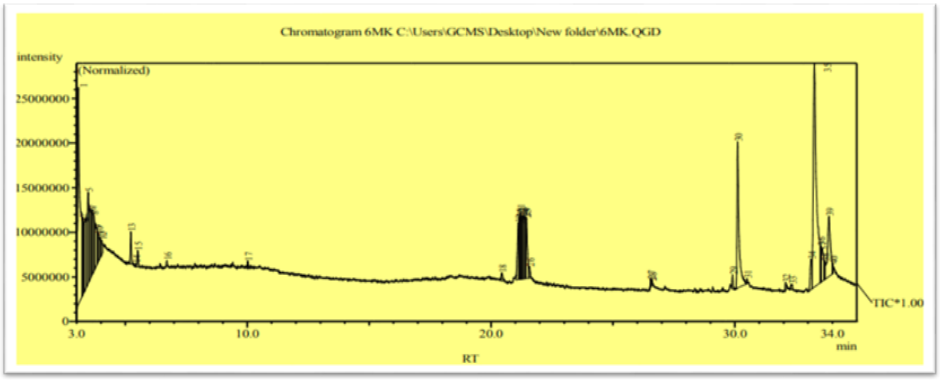

Figure 5: Chromatogram of *Punica granatum* peel extract by using GC-MS technology

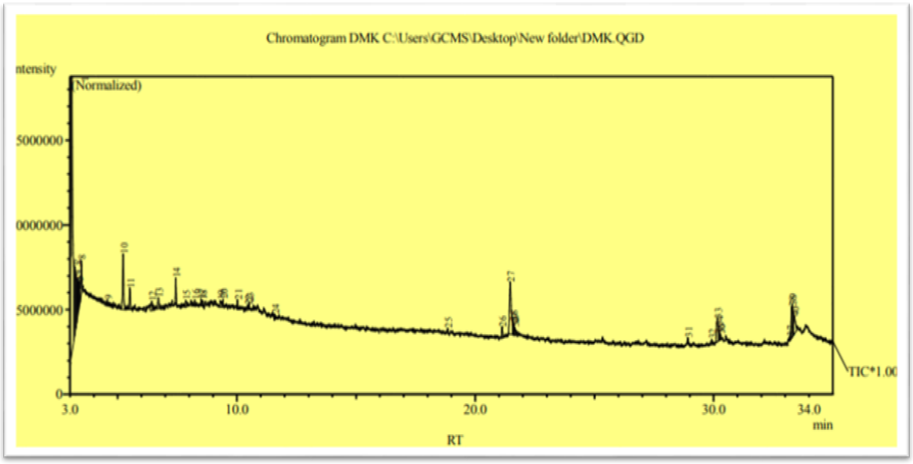

Figure 6: Chromatogram of mixture extracts (*Ziziphus spina - christi* leaves and *Eucalyptus camaldolehsis* leaves) by using GC-MS technology

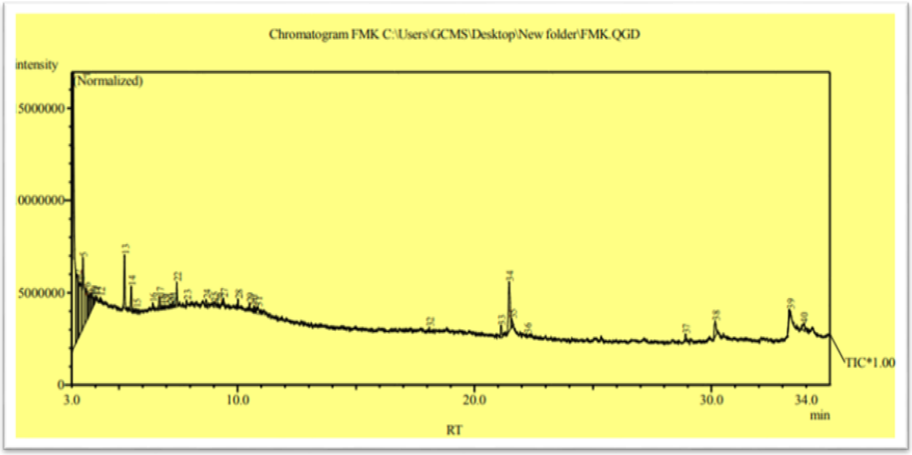

Figure 7: Chromatogram of mixture extracts (*Eucalyptus camaldolehsis* leaves and *Punica granatum* peel) by using GC-MS technology

The study showed that the peaks demonstrated by the GC-MS analysis curves indicate the nature of the presented chemical compounds in the extracts, as this technique works to isolate the compounds at different times, and then diagnose them by determining their composition, molecular weight, and chemical formula, and they are diagnosed through the library data stored in the device.

The new chemical compounds were identified by using Gc-Ms technology for a mixture of *Ziziphusspina-christi* leaves extracts with *Punica granatum* peel. The chemical composition, molecular formula, and biological activity of the new compounds were indicated as in Table 3.

Likewise, the biological activity, chemical composition, and chemical formula of the new compounds listed in Table 4, which resulted from mixing *Ziziphusspina-christi* leaves extract with *Eucalyptus camaldulensis* leaves extract were studied. These new compounds were detected by using Gc-Ms technology.

The biological activity, chemical composition, and chemical formula listed in Table 5 were also studied for the new compounds resulting from mixing *Eucalyptus camaldulensis* extract with *Punica granatum* peels extract. These new compounds were detected by using the Gc-Ms.

Table 3. Biological significance, chemical composition, and chemical formula of new compounds resulting from mixing *Ziziphusspina-christi* leaves extract with *Punica granatum* peel extract

No.	Name of the Compound	Biological activity
1	Ethanol, 2-nitro- Or 2-nitroethanol	Toxic effect as the vapor or mist of this compound irritates the eyes, upper respiratory tract, and mucous membranes [15].
2	2-isopropoxy ethyl amine Or 2-Aminoethyl Isopropyl Ether	Used to make medical narcotic and pesticide herbicide [16].
3	Methoxy acetic acid iso propyl ester or 1-methoxypropan-2-yl acetate	Anti-cancer.Methoxyisopropyl acetate is used in surface coatings, and cleaners [17].
4	acetonitrite,bromo or 2-bromoacetonitrile	Carcinogens [18]
5	Benzene1,2,4-trimethyl	It causes rapid breathing but has no effects on the central nervous system [19].
6	Acetic acid,cyano	It is used as a chemical medium for malonic acid. Diethyl Malonate Pharmaceuticals. It is used to produce the fungicide cymoxanil and to treat dextromethorphan cough [20].
7	3-Isothiazolecarboxamide, 4-amino	It is used as an antiviral drug. The isothiazole ring has also pharmacological activity. Likewise, the effect of structural

		modifications in the carboxylic group. Derivatives on their biological activity [22].
8	1-Adamantanemethylamine, .alpha.-methyl-	Antiviral agents used in the prophylaxis or therapy of virus diseases. Some of the ways they may act include preventing viral replication by inhibiting viral DNA polymerase; inhibiting viral protein synthesis. It is used as an anti-infection. It also works as antimicrobial agents and chemotherapy [22].
9	1-(4 -Acetamidoanilino)-3,7-dimethylbenzo [4,5] imidazo [1,2-a]pyridine-4 -carbonitrile	Unknown activity
10	Methoxyacetic acid, octyl ester	Unknown activity
11	Benzene, (1,1,2-trimethylpropyl	Unknown activity
12	Benzene, 1,3-bis(1-methylethyl	It is used in various products and industries (e.g. in cosmetics, chemical manufacturing, production of metals, etc.).This compound is considered a risk, as continuous exposure to the vapors of this compound causes the early cancer cases and inhibits growth [23].
13	Hexanal	Flavoring agents. Anti-fungal agents' substances that eliminate fungi. Well used as insecticides designed to combat insects harmful to humans. Anti-microbial agents and anti-bacterial agente [24].
14	2-Propyl-tetrahydropyran-3-ol	Anti-angiogenic effect [25]
15	4-Chloro-3-n-hexyltetrahydropyran	Unknown activity
16	Cis -bicyclo[4.4.0] decan-1-ol-3-one	Antibacterial activity of Eryngium maritimum L [26].

Table 4: Biological significance, chemical composition, and chemical formula of new compounds resulting from mixing *Ziziphusspina-christi* leaves extract with *Eucalyptus camaldulensis* leaves extract

No.	Name of the Compound	Biological activity
5	Bicyclo[2.1.1]hex-2-ene, 2 -ethenyl-	Antifungal [27].
7	Benzene, 1-ethyl-3-methyl-	Antimicrobial Activities, antioxidant, and antimicrobial agents [28].
8	Methoxyacetic acid, 2-pentyl ester	Antimicrobial activity is reported [29].
9	4 -Amino-2-oxy -furazan-3 -carboxylic acid	Unknown activity
11	Acetic acid, ethoxyhydroxy-, ethyl ester	Unknown activity
12	(3-Methyl-oxiran-2-yl)-methanol	Unknown activity
13	1,3 -Cyclopentadiene,1,2,3,4-tetramethyl -5-methylene-	Unknown activity
14	7,10-Epoxytricyclo[4.2.1.1(2,5)]decane, 1-trimethylsilyl-	Unknown activity
15	Benzene, 1,4-bis(1-methylethyl)	Antioxidant, Inhibitors of the interaction of thyroid hormone [30].
23	2-(4,5-Dihydro-3-methyl -5-oxo-1-phenyl-4 -pyrazolyl)-5-nitrobenzoic acid	It is used as pesticide, miticide, and weed killers, it also possess good antibacterial [31].
25	Acetic acid, cyano-	It is used as a chemical medium for malonic acid. Diethyl Malonate Pharmaceuticals It is also used to produce the fungicide simoxanil and dextromethorphan cough treatment [32].

Table 5: Biological significance, chemical composition, and chemical formula of new compounds resulting from mixing *Eucalyptus camaldulensis* extract with *Punica granatum* peels extract

No.	Name of the Compound	Biological activity
1	(S)-Isopropyl lactate	It has a toxic effect that causes muscle weakness upon intramuscular injection [33].
2	1-Octanethiol	Accelerators, activators, oxidation agents, reducing agents, etc. This compound have a toxic effect may be fatal if inhaled, ingested, or absorbed through skin [34].
3	Butanoic acid, 3-hydroxy	It acts as an inhibitor of the hepatitis C virus (HCV). It is also used by the brain as a source of energy during fasting in humans or when blood glucose is low used in the synthesis of polyvinyl-co-unsaturated acids resins, pharmaceutics [35].
4	2,4-Pentanediol	Inhibitors of death of the malaria parasite plastid is also used to screen green cytotoxicity to monitor cytotoxicity in cells [36].
5	Ketone, methyl 4-pyridyl, O-acetyloxime	Unknown activity
6	Benzene, 1-methyl-4-(1-methylethyl)	Used as flavoring agents, it also works to reduce acute lung injury caused by lipopolysaccharide (LPS) *in vivo*. Anatomic examination of lung tissue has shown that treatment with this compound reduces pneumonia and has a protective effect on the lung. Non-confidential commercial and consumer uses of p-cymene include polishes and sanitation goods, soaps, detergents, and other [37].
7	Diglycerol	Has unique functions as a basic component of membranes, an intermediate in lipid metabolism [34].
8	1,3,5 -Cycloheptatriene, 3,7,7 -trimethyl	It treats dementia and cognitive deficits in Parkinson's disease, and / or lack of learning, and memory in Parkinson'sdisease [38].
9	2-Thiazolidinethione	It has various biological activities, such as bactericidal, pesticide, anti-convulsants, anti-tuberculosis, anti-flatulence, painkillers, anti-parasites, and herbicides, it also has the strong anti-HIV and anti-cancer [39].
10	Cyclopentanemethanol, .alpha.-methyl	Anti-cancer Drug [34].
11	Cyclohexanepropanol, .alpha.-methyl	Against several bacteria and fungi [30].
12	Trans -Z-.alpha.-Bisabolene epoxide	Biological activity and anti-microbes [33].
13	Clonitazene	Unknown activity
14	2,4-Decadien-1-ol	Identification of antagonists of small molecules of thyroid receptors, an assay of small molecule stimuli of vitamin D [39].
15	Threitol, 2-O-octyl-	Unknown activity

Conclusion

It can be mentioned that the preparation of three mixture extracts prepared by mixing (1:1) two of each of the pure extracts separately, demonstrated us new chemical compounds that differ from the chemical compounds appeared in the pure extracts, by conducting chemical analysis by using the GC-MS technique. These new chemical compounds were identified in each of the prepared extract blends and their benefits are theoretically studied. It should be noted that this these compounds are naturally present in other sources or may be chemically prepared in the laboratory, but they are not presented in the original extracts from which they were prepared, and therefore it is a simple and new way to mix pure extracts and prepare new chemical compounds with different biological activity.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

There are no conflicts of interest in this study.

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