Preliminary Phytochemical and Screening of Biocomponents by GC-MS Technique in *Myrtuscommunis*L. Plant Flowers

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Abstract

Phytochemical components of *Myrtuscommunis* L. flowers have been evaluated using GC-MS. The chemical compositions of *M.communis* L. flowers were investigated using Shimadzu Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched by the National Institute of Standards and Technology (NIST) library. The qualitative analysis of ethanolic extract of *M.communis* L. flowers showed that alkaloid, tannin, phenol, flavonoids, terpenoids, sapanoin, anthraquinones, protein and quinones present in ethanolic extract. GC-MS analysis of ethanolic extract of *M.communis* L. flowers revealed the existence of acetic acid, camphor, Bicyclo[2.2.1]heptane, 2-methoxy-1,7,7-trimethyl-, 2-Butenoic acid, 2-methyl-, (Z)-, 1-Hexadecanol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, 1,3-Cyclopentadiene, 5,5-dimethyl-2-ethyl-, Diazoacetic acid, 2-isopropyl-5-methylcyclohexyl ester, 3,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-, 3-Oxabicyclo[4.1.0]heptan-2-one, 4,4,7,7-tetramethyl-, 12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, 3-Buten-2-one, 4-(2-hydroxy-2,6,6-trimethylcyclohexyl)- and thujone. The quantitative determination of an ethanolic extract of *M. communis*L. flowers contains high concentrations of phenol (7.84 ± 0.69 mg/gm), flavonoids (5.23 ± 0.56 mg/gm), tannin (2.04 ± 0.43 mg/gm), and saponins (1.83 ± 0.49 mg/gm). The results of this study offer a platform of using *M.communis*L. an herbal alternative for various diseases.

Botany Classification QK 710-899

Keywords: *M. communis*L., Phytochemical components, GC-MS.

Introduction

Myrtuscommunis L. or myrtle is an evergreen perennial shrub and fragrant. Its stems are very numerous, ramified and branches are of leaves near one another and dispersed with a gray coverage and white flowers being seen in humid and semi- humid areas [1, 2]. Great activities have been done regarding the pharmaceutical effects of this plant. [3] Through examining antifungal effects of myrtle plants leaf extract in Roudbar on some of saprophytes and dermatophytes fungi in in-vitro condition came to this conclusion that the hydro alcoholic extract of myrtle is effective on *Trichophaytonmenthagrophaytis*, *Epidermophaytonphelokozom* and *Michrospormkanis* fungi. In another research, [4] studied antibacterial effect of myrtle's essential oil and concluded that this plant is of antimicrobial characteristics. In the other research also, the antibacterial, antioxidant effect and sterilization activity of this plant has been examined [5, 6, 7].

Medicinal plants are various plants used in herbalism and thought by some to have medicinal properties. Medical Plant constitutes an important therapeutic aid in alleviating ailments. Almost 80% of the world populations, particularly in the third world are fully dependent on medicinal plants for meeting their health care needs, the herbal medicines today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. In the primeval times, the Indian sagacious held the view that herbal medicines are the only resolution to treat numeral health related problems and diseases. It is becoming more that stream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in the treating and preventing the disease, increasing interest in herbal products has today accelerated the growth of medicinal plant based industries [8].

Traditional medicinal usage of herbs by humans, however imperfect and "unscientific" by modern standards, is the result of countless trial-and-error tests that people have conducted, and so traditional usage points the way to natural therapeutic usage. As we later stress, however, "natural" does not necessarily mean "safe", some herbal products are extremely effective, but so dangerous that they should only be used in the hands of skilled medical professionals. Others, however, are sufficiently safe that they can be used by laypeople to help prevent or alleviate minor health problems, sometimes the herbal drugs are preferable, but as we stress throughout this work, qualified medical personnel should always be consulted [9].

Within a decade, there were a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation, identification and structure determination of phytochemicals [10].

The aim of study

Is to determine of organic compounds in the *M. communis*L.flowers extract with the aid of GC-MS technique, which may provide an insight in its use in tradition medicine.

Materials and Methods

- **Plant material**: Flowers of *M. communis*L. were selected to screen its biopotentials based on its traditional usage. The fully mature of plantflowers were collected in May 2015 from a single herb in Al-Diwaniya city/ Iraq and care was taken to select healthy flower.
- **Preparation of extract**: *M. communis*L. flowers collected and washed under running tap water and dust was removed from the flowers. The flowers were dried at room temperature for 15 days and coarsely powdered by the use of small grinder. The powder (2 g) was extracted with 70% ethanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *M. communis*L. flowers extract was stored in refrigerator until used. Chemical tests were carried out on the alcoholic extract using standard procedures to identify the preliminary phytochemical screening following the methodology of [11, 12, 13].
- **Quantitative determination of the chemical constituency:**
  - ✓ Preparation of fat free sample: 2 g of the sample was defatted with 100 ml of diethyl ether using a Soxhlet apparatus for two hours.
  - ✓ Determination of total phenols by spectrophotometric method [14]: The fat free sample was boiled with 50 ml of ether (95%) for the extraction of the phenolic component for 15 min. 5 ml of

the extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol (95%) were also added. The samples were made up to mark and left to react for 30 min for colour development, this was measured at 505 nm.

- ✓ Flavonoid determined by the method [15]: 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whitman filter paper No 42 (125 µm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.
- ✓ Tannin determination by the method [16]: 500 mg of the sample was weighed by into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipette out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.
- ✓ Saponins determination [17]: The samples were ground and 20 g of each were put into a conical flask and 100 cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4h with continuous stirring at about 55 ºC. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at about 90 ºC. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a weight constant.

- **GC-MS method:** GC-MS analysis was carried out on a Shimadzu GCMS-QP2010Ultra system comprising a AOC-20i (Japanese-made) in auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID × 1 µMdf, composed of 100% Dimethyl polysiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 240 ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 100 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200 ºC, then 5 ºC/min to 280 ºC, ending with a 9min isothermal at 280 ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min. min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMassVer 5.2.0 [18]. Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Results and Discussion

Table 1 and 2 represent the phytochemical constituents present in *M. communis* L. flowers. The qualitative analysis of ethanolic extract of *M. communis* L. flowers contain alkaloid, tannin, phenol, flavonoids, terpenoids, sapanoin, anthraquinones, protein and quinones present in ethanolic extract.

The quantitative determination of an ethanolic extract of *M. communis* L. flowers contains high concentrations of phenol (7.84 ± 0.69 mg/gm), tannin (4.10 ± 0.43 mg/gm), flavonoids (5.23 ± 0.56 mg/gm) and saponins (4.10 ± 0.49mg/gm). The results of the study demonstrated that the ethanolic extraction had a higher content of the phytochemicals. Phenols are the secondary metabolites that are

ubiquitously present in plants. They have been suggested to play a role in the antioxidant function. Phenolic compounds have antioxidant properties because of their ability to scavenge free radicals. The phenolic compounds of plant origin showed their anti-oxidative effect by various mechanisms, including their ability to scavenge free radicals or activate various antioxidant enzymes and inhibit oxidizes [19].

Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. Flavonoids are 15 carbon compounds generally distributed throughout the plant kingdom. Some isoflavones widely used in insecticides. They might also play a role in disease resistance. Some flavonoids such as quercetinand rutin, are known to support human health by serving anti-inflammatory, antihistaminic and antiviral agents [20]. Flavonoid compounds exhibit inhibitory effects against multiple viruses. Numerous studies have documented the effectiveness of flavonoids, such as glycyrrhizin and chrysin [21] against HIV. Flavonoids are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity [22]. Flavonoids have been referred to as nature's biological response modifiers, because of inherent ability to modify the body's reaction to allergies. It possesses various pharmacological roles including anti-allergic, anti-inflammatory, cardio-protective, anti-microbial and anti-cancer activities [21].

Thirteen compounds were identified in *M. communis* L. flowers by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) are presented in (Fig. 1, 2-1, 2-2, 2-3, 2-4). The GC-MS method confirms that *M. communis* L. contain Acetic acid, Camphor, Bicyclo[2.2.1]heptane, 2-methoxy-1,7,7-trimethyl-, 2-Butenoic acid, 2-methyl-, (Z)-, 1-Hexadecanol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, 1,3-Cyclopentadiene, 5,5-dimethyl-2-ethyl-, Diazoacetic acid, 2-isopropyl-5-methylcyclohexyl ester, 3,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-, 3-Oxabicyclo[4.1.0]heptan-2-one, 4,4,7,7-tetramethyl-, 12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)]-, 3-Buten-2-one, 4-(2-hydroxy-2,6,6-trimethylcyclohexyl)- and thujone. The biological activities listed (Table 3) are based on [23].

The results confirm the presence of constituents which are known to exhibit medicinal value as well as physiological activities. The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit. Therefore, the date generated from these experimental has provided the chemical basis for the wide use of this plant as therapeutic agent for treating various ailments. The results of this study offer a platform of using *M. communis* L. flowers as herbal alternative for various diseases including diabetic, cancer, cardiovascular…etc.

Table 1: Preliminary phytochemical screening of *M. communis* L. flowers ethanolic extract

S. No	Secondary metabolites	Ethanol flowers extract of *M. communis* L.
1	Alkaloid	+
2	Tannin	+
3	Phenol	+
4	Flavonoids	+
5	Sterol	+
6	Terpenoids	-
7	Saponins	-
8	Anthraquinones	+
9	Protein	+
10	Quinones	+

(+) Presence; (-) Absence

Table 2: Quantitative analysis of ethanolic extract of phytochemical in *M. communis* L. flowers (mg/g)

S. No	Secondary metabolites	Mean ± SD
1	Phenol	7.84 ± 0.69
2	Flavonoids	5.23 ± 0.56
3	Tannin	2.04 ± 0.43
4	Saponins	1.83 ± 0.27

Table 3: Biological activity of *M. communis* L. flowers extract components identified by GC-MS

S. No	Name of the Compound	Suggested biological activity
1	Camphor	Antimicrobial,

		Anti-inflammatory
2	Bicyclo[2.2.1]heptane, 2-methoxy-1,7,7-trimethyl-, 2-Butenoic acid, 2-methyl-, (Z)-, 1-Hexadecanol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol,	Anti-tumor, Analgesic, Antibacterial, Anti-inflammatory, Fungicide
3	1,3-Cyclopentadiene ,5,5-dimethyl-2-ethyl-, Diazoacetic acid, 2-isopropyl-5-methylcyclohexyl ester,	Antimicrobial, Antifouling
4	3,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-, 3-Oxabicyclo[4.1.0]heptan-2-one, 4,4,7,7-tetramethyl-, 3-Buten-2-one,4-(2-hydroxy-2,6,6-trimethylcyclohexyl)- and thujone.	Antiulcer, Anti-phlogistic effects, Antimicrobial, Antioxidant, Anti-inflammatory, Promotes wound healing

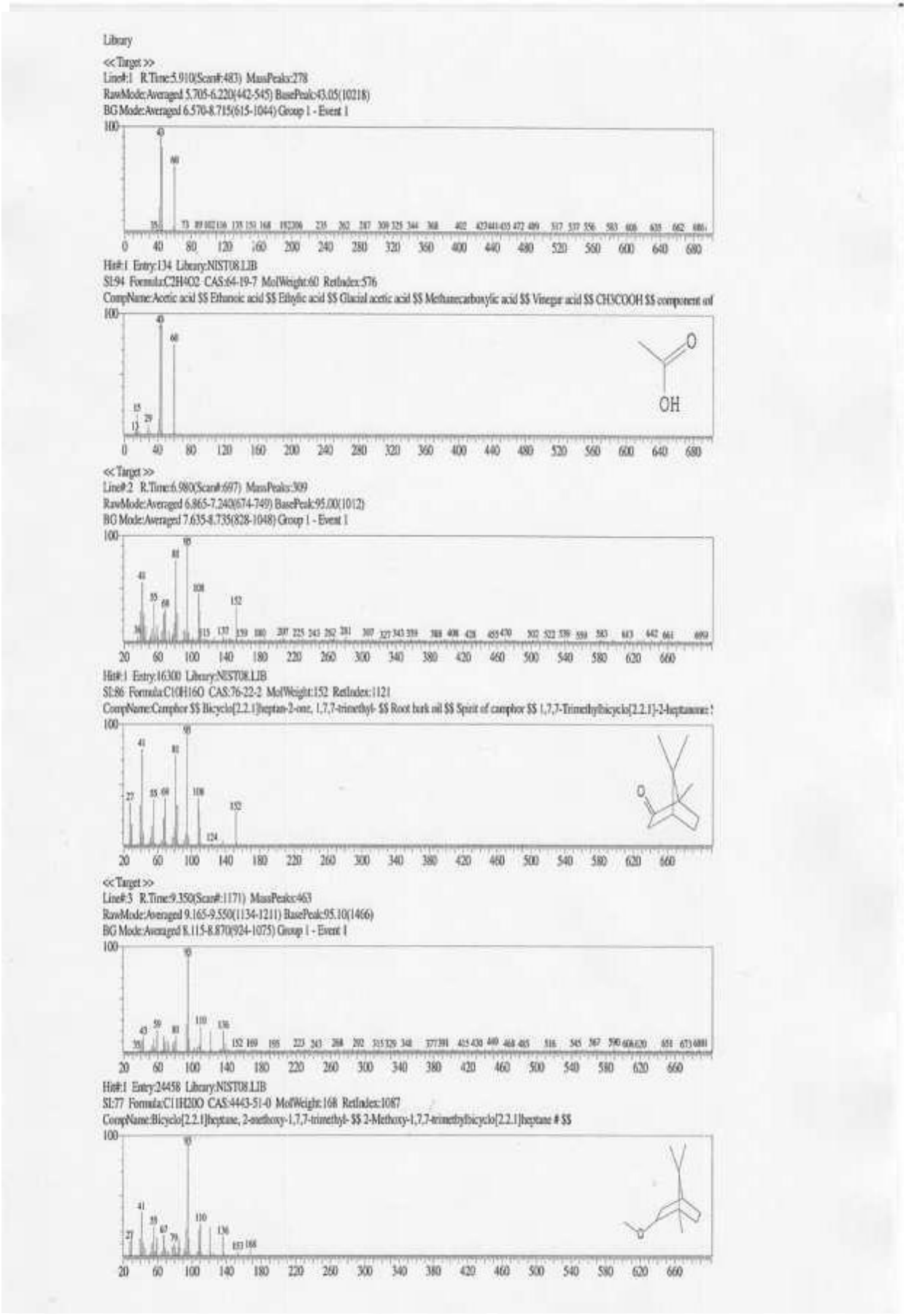
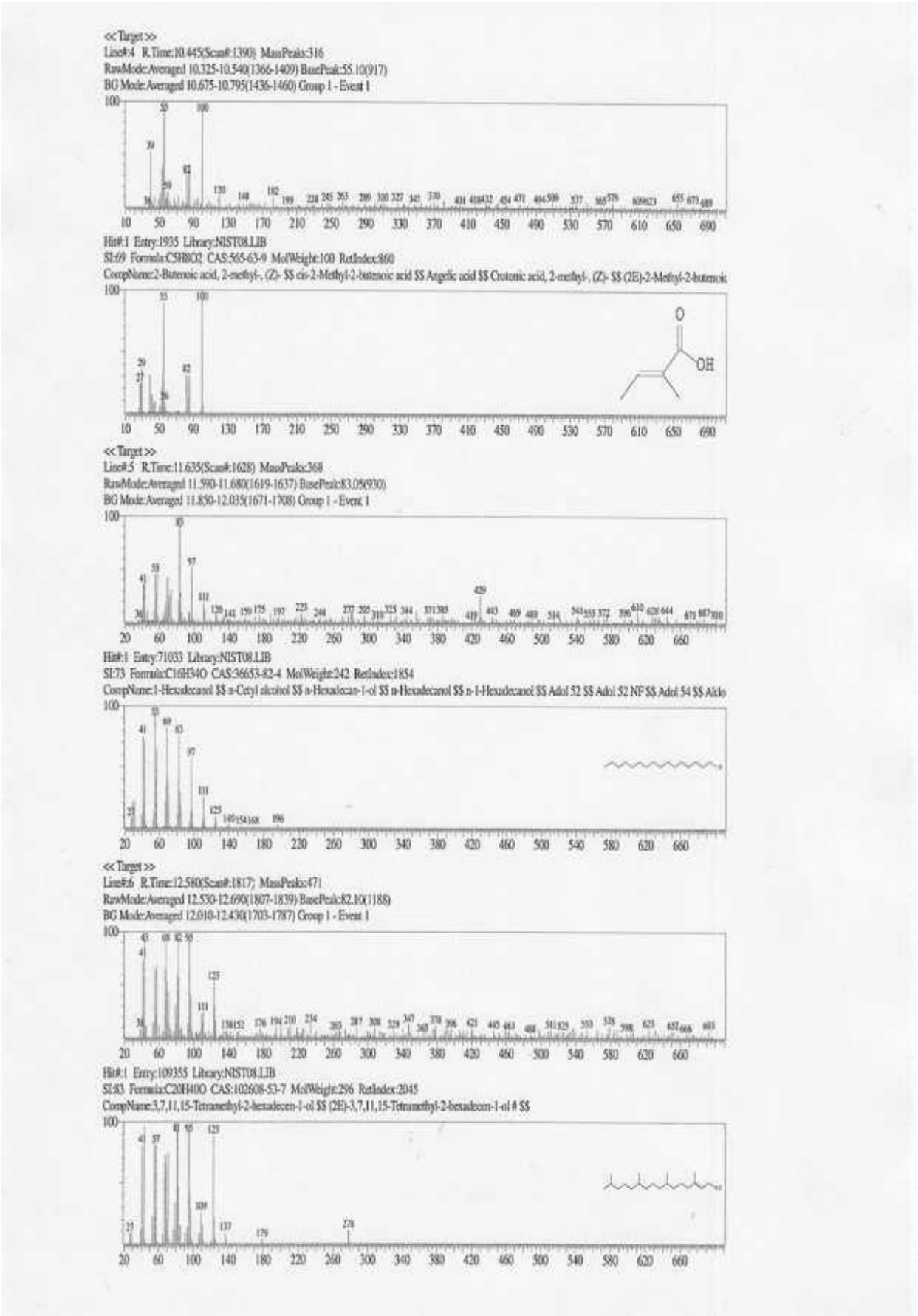
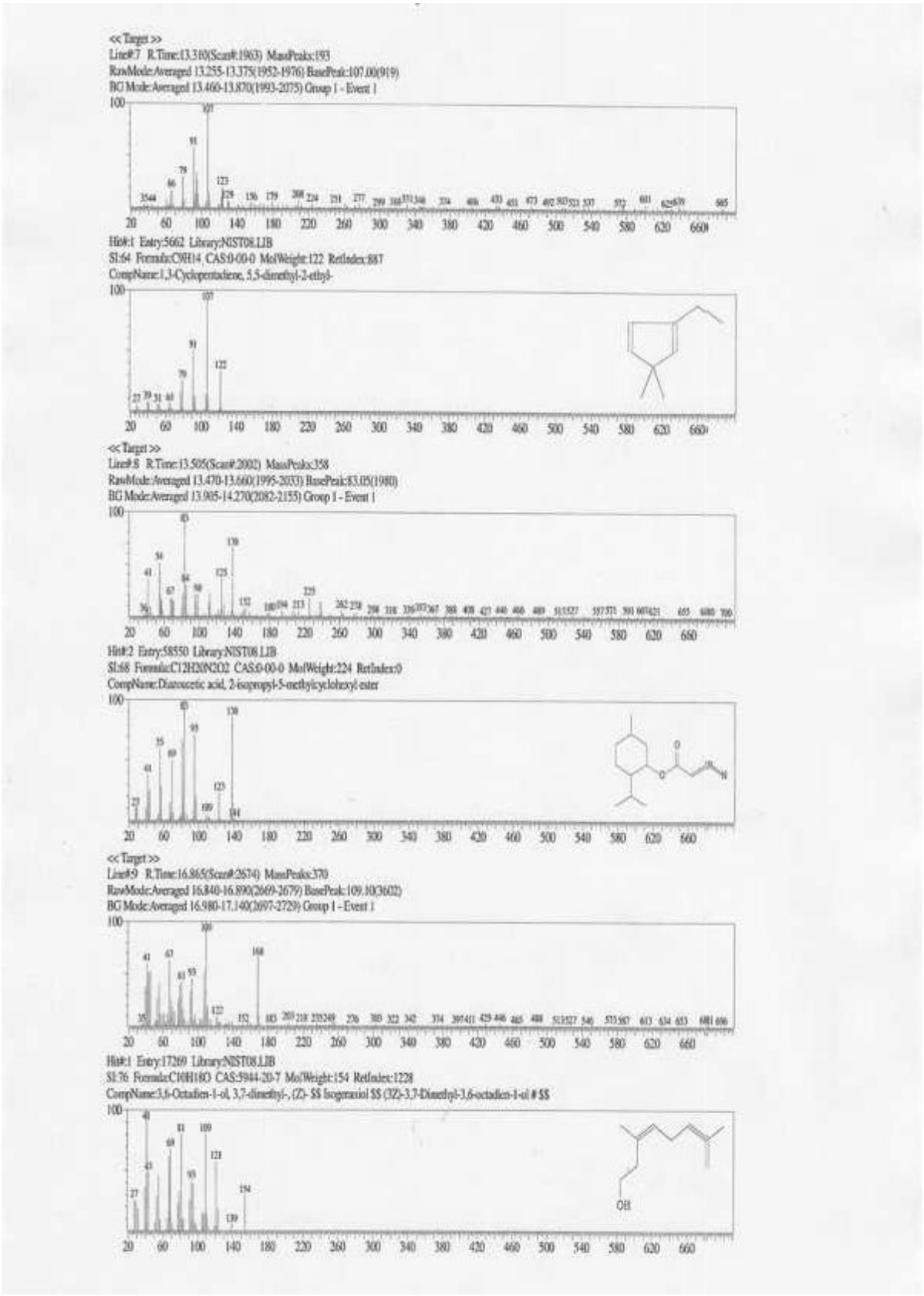


Fig 1: Chromatogram obtained from the GC-MS with the extract of *M. communis* L. flowersFig 2-1:Chemical components obtained from the GC-MS with the extract



of *M. communis*L. flowers by NISTprovidedFig 2-2: Chemical components obtained from the GC-MS with the extract of *M. communis* L. flowers by



NISTrovidedFig 2-3: Chemical components obtained from the GC-MS with the extract of *M. communis*L. flowers by NISTprovided

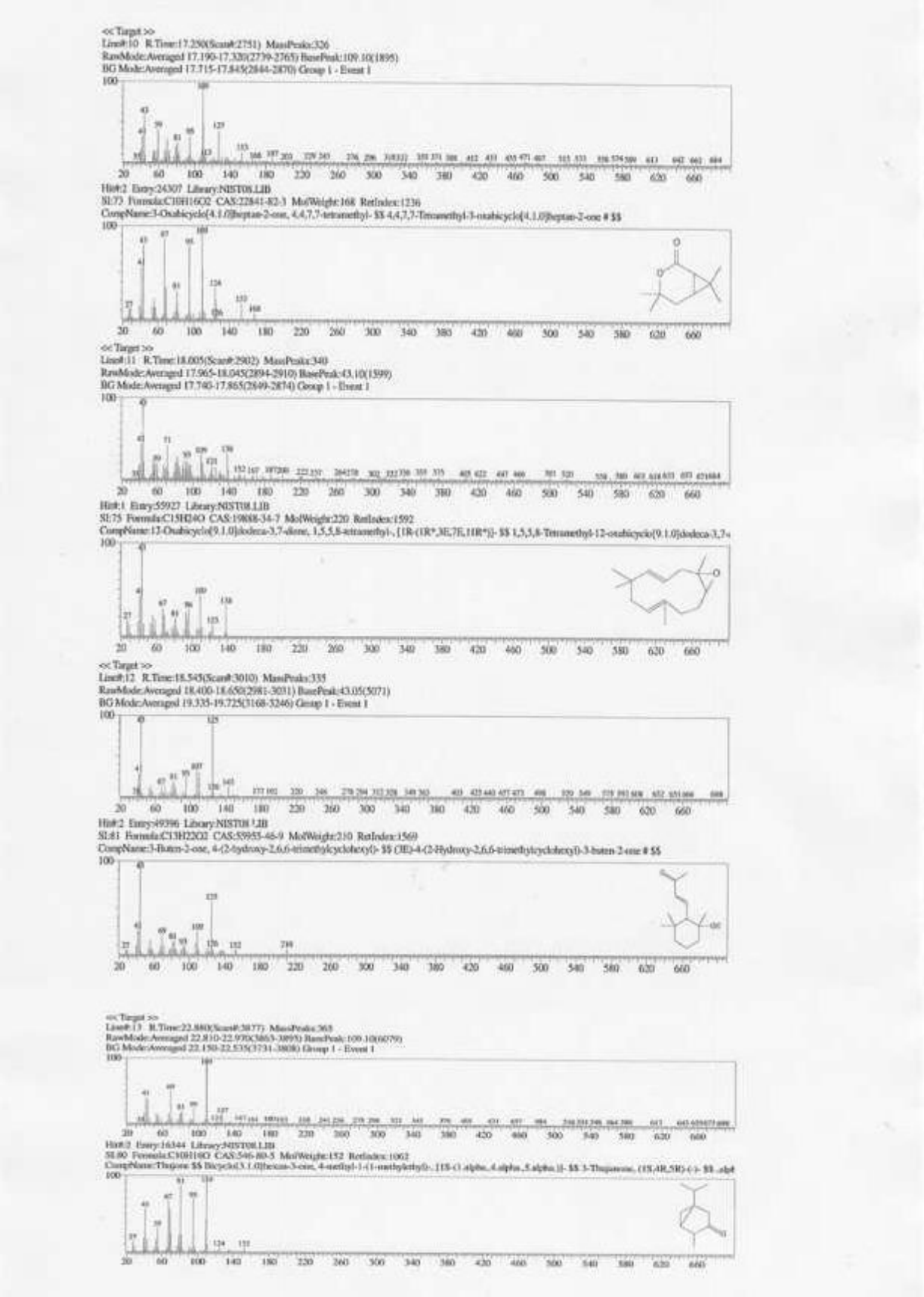


Fig 3-4: Chemical components obtained from the GC-MS with the extract of *M. communis*L. flowers by NISTprovided

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الكشف الأولي للمركبات الكيموحيوية النباتية وتحليل المكونات الحيوية بواسطة تقنية GC–MS في أزهار نبات الآس *Myrtuscommunis* L.

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الخلاصة

تم في الدراسة الحالية تعيين المكونات الكيموحيوية النباتية في أزهار نبات الآس *Myrtuscommunis* L. باستعمال جهاز Shimadzu Gas Chromatography–Mass Spectrometry وقورنت أطياف المركبات لمستخلص الأزهار التي تم الحصول عليها مع يقابلها من أطياف للمركبات الموجودة في مكتبة المعهد الوطني للقياس كنولوجيا(NIST). وأظهر التحليل النوعي لمستخلص الإيثانول للأزهار وجود المواد: alkaloid, tannin, phenol, flavonoids, terpenoids, sapanoins, anthraquinones, protein, quinones. وأظهر التحليل الكمي لمستخلص الأزهار بواسطةGC-MS وجود المواد: acetic acid, camphor, Bicyclo[2.2.1]heptane, 2-methoxy-1,7,7-trimethyl-, 2-Butenoic acid, 2-methyl-, (Z)-, 1-Hexadecanol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, 1,3-Cyclopentadiene, 5,5-dimethyl-2-ethyl-, Diazoacetic acid, 2-isopropyl-5-methylcyclohexyl ester, 3,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-, 3-Oxabicyclo[4.1.0]heptan-2-one, 4,4,7,7-tetramethyl-, 12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, 3-Buten-2-one, 4-(2-hydroxy-2,6,6-trimethylcyclohexyl)- and thujone. كما أن التقدير الكمي للمواد في مستخلص الأزهار الكحولي كشف عن تراكيز عالية من الفينول (7.84 ± 0.69 ملغم/ غم) و الفلافونويد (5.23 ± 0.56 ملغم/غم) وقليلة من التانين (2.04 ± 0.43 ملغم /غم) و الصابونين (1.83 ± 0.49 ملغم/غم). موفرة بذلك نتائج الدراسة الحالية من إستعمال نبات الآس منصة عشبية بديلة للأمراض المختلفة.

الكلمات المتاحية: نبات الآس, المكوناتالكيموحيوية النباتية,GC-MS.