

The Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Seed Oil of Retama Raetam

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Abstract:

One of the most accurate techniques for identifying the various secondary metabolites present in plant oils is gas chromatography-mass spectrometry (GC-MS). The seed oil of Retama raetam was analyzed by GC-MS to detect various compounds present in it. The GC-MS analysis revealed 47 chemical compounds. (4-Bromophenyl) bis (2, 4-dibromophenyl) amine (54.78 min) is the highest retention time chemical compound, followed by 2, 7, 12, 17-tetrabrom (allàs) cyclotetrathiophen (5.17 min) as the lowest retention time chemical compound. The most abundant phytochemicals identified in seed oil of Retama raetam are 9,12-octadecadienoic acid (Z,Z), methyl ester (42.86%), dodecanoic acid, 3,7,11 trimethyl, methyl ester (19.42%), and 9-octadecenoic acid (Z,Z), methyl ester (13.77%), respectively. In vitro antimicrobial screening of the seed oil of Retama raetam against Gram-positive (St. aureus ATCC 25923) and Gram-negative (E. coli ATCC 25922) and Ps. aureginosa ATCC 25850) bacteria was evaluated in this study, and the obtained results revealed activity only against St. aureus, with no activity recorded against E. coli or Ps. aureginosa. Fungi (Candida albicans ATCC 10213) was also evaluated, with no activity recorded for different concentrations of the seed oil of Retama raetam (100%, 50%, and 25%). Thus, it indicates that the seed oil of Retama raetam has activity only against St. aureus. The resultant antibacterial activity exhibited by the seed oil of Retama raetam is related to the presence of phytochemicals identified in the seed oil of Retama raetam, which were identified by using GC-MS and reported to have antimicrobial and antibacterial activity.

Keywords: Chromatography-Mass Spectrometry (GC-MS), Seed Oil, Retama Raetam

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تحليل كروماتوغرافيا الغاز - مطياف الكتلة (GC-MS) لزيت بذور ريتاما رايتام

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الملخص

واحدة من أكثر التقنيات دقة لتحديد مختلف المستقلبات الثانوية الموجودة في الزيوت النباتية هي قياس طيف كتلة كروماتوغرافيا الغاز (GC-MS). تم تحليل زيت بذور ريتاما رايتام بواسطة GC-MS للكشف عن المركبات المختلفة الموجودة فيه. كشف تحليل GC-MS عن 47 مركبًا كيميائيًا (4-Bromophenyl-مكررا (2، 4-ثنائي بروموفينيل) أمين (54.78 دقيقة) هو المركب الكيميائي الأعلى وقت الاحتفاظ، يليه المركب الكيميائي 2، 7، 12، 17-رباعي بروم (الأس) cyclotetrathiophen (5.17 دقيقة) باعتباره أقل مركب كيميائي وقت الاحتفاظ. والمركبات النباتية الأكثر وفرة التي تم تحديدها في زيت بذور ريتاما رايتام هي 9 و12-ثمانية الكيادينويكيد (Z, Z) واستر الميثيل (42,86%) وحمض الوديكانويك 3,7,11 وثلاثي ميثيل واستر الميثيل (19,42%) وحمض 9-ثمانينيك (Z, Z) على التوالي وميثيل 13,77%. في المختبر، فحص زيت بذور ريتاما ضد إيجابي الغرام) سانت أوريوس ATCC 25923 وسلبلي الغرام (E. Coli ATCC 25922) و P. aureginosa ATCC 25850 تم تقييم البكتيريا مع عدم وجود نشاط فقط في هذه الدراسة، و كما تم تقييم الفطريات (المبيضات البيضاء) ATCC 10213، مع عدم تسجيل أي نشاط لتركيزات مختلفة من زيت بذور ريتاما (100%، 50%، 25%). وبالتالي، فإنه يشير إلى أن زيت بذور ريتاما رايتام له نشاط فقط ضد القديس أوريوس. يرتبط النشاط المضاد للبكتيريا الناتج عن زيت بذور ريتاما رايتام بوجود مركبات نباتية تم تحديدها في زيت بذور ريتاما رايتام، والتي تم تحديدها باستخدام GC-MS وأفيد عن وجود نشاط مضاد للميكروبات ومضاد للبكتيريا.

الكلمات المفتاحية: طيف الكتلة الكروماتوغرافية (GC-MS)، زيت البذور، ريتاما رايتام.

Introduction

Medicinal plants are considered as a rich resource of ingredients which can be used in drug development either pharmacopoeial, non- pharmacopoeial or synthetic drugs. A part from that, these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants are considered as important source of nutrition and as a result of that they are recommended for their therapeutic values [1]. The importance of medicinal Plant species was attributed to the presence of huge number of active ingredients such as alkaloids, phenols, tannins, cryogenics, glycosides, terpenoids. These ingredients have been used and found effective as sweeteners, anti-infective and anti-bacterial. For instance, the bark of *Alstonia boonei* contains alkaloids and achistamine, which are useful in the treatment of fever, dizziness and high blood pressure. Ginger (*Zingiber officinale*) and Garlic (*Alliumsativum*) are spicy additions to food that has long been used to maintain human health. It is not an exaggeration to say that medicinal plants have a great role to play in sustainable human health [2]. The stage of identification of biomedical compounds of medical importance from the old was began, and gradually developed. These biomedical compounds classified as primary and secondary metabolites. Primary metabolites include chlorophyll, cane sugar, starch, camphor and benzoic acid had long been known, as their preparation was extremely simple, also complex mixtures such as fats, fixed oils, volatile oils, tars and resins had been prepared and used. Secondary metabolites like alkaloids, sterols, terpenes, flavonoids, saponins, glycosides, cyanogenics, tannins, resins, lactones, quinines, volatile oils where synthesized and stored inside plants, which lead to the progress occurred more rapid in 19th century [3]. There are various examples of development of new drugs from the plant sources. Morphine was isolated from opium produced from cut seed pods of the poppy plant (*Papaver somniferum*) approximately 200 years ago. Pharmaceutical research expanded after the Second World War to include massive screening of microorganisms for new antibiotics, inspired by the discovery of penicillin. Few drugs developed from natural sources have undoubtedly revolutionized medicine, like antibiotics (e.g. penicillin, tetracycline, and erythromycin). Antiparasitic (e.g. avermectin), antimalarials (e.g. quinine, artemisinin), lipid control agents (e.g. lovastatin and analogs), immunosuppressants for organ transplants (e.g. cyclosporine, rapamycins), and anticancer drugs (e.g. paclitaxel, irinotecan) [4]. Since the beginning of natural herbal medicine, oils are one of the most ancient forms of folk medicine. Herbal oils have been used since ancient times for their preservative and medicinal properties, and to impart aroma and flavor to food. Hippocrates, sometimes referred to as the “father of medicine,” prescribed perfume fumigations. The pharmaceutical properties of aromatic plants are partially attributed to essential oils. The term “essential oil” was used for the first time in the sixteenth century by Paracelsus von Hohenheim. The historical Persian medical and pharmacological manuscripts are the first to describe the importance of using oils for therapeutic purpose and their forms of administrations (i.e. systemic or topical). Massage by herbal oils was the culture of traditional Chinese, Indian and Egyptian medicine. As frequent application of herbal oils to the newborn skin thought improve skin condition, protect skin from injury and infections, and also keep the moisture content in the skin. It has been observed the massaging with Sesame oil for newborn baby, improves sleep pattern and growth of limbs [5],[6],[7]. The medicinal values of essential oils have been recognized for many plants, like: *Thymus schimperi* leaves oil has anthelmintic, antibacterial, and antifungal activity, leaves oil of *Eucalyptus globulus* can boost the immune system and is helpful in cases of chicken pox, colds, flu, measles, and infectious diseases, *Matricaria chamomilla* flower oil is good for skin care, acne, allergies, boils, burns, eczema, inflamed skin conditions, and wounds and used for infection, and *Rosmarinus officinalis* leaves oil possesses antitumor and anti-inflammatory

actions and antimicrobial activity [8]. In recent years, essential oils have received increasing attention as they exhibit significant antibacterial, antifungal, and antioxidant activities [9]. In the earlier time, the process of isolation and identification the chemical identity of these bioactive constituents was time wasting. Recently, availability of new techniques such Gas Chromatography or gas chromatography-mass spectroscopy (GC-MS) are most exclusively for the qualitative analysis of the volatiles [10]. Based on importance and extent of medicinal and toxic effects of secondary constituents, we have done a research on seed oil of Retama Raetam, medicinal plant with biologically active constituents which will be presented later. The aim of this study to investigate the chemical composition of the R. Raetam seed oil and to evaluate the antimicrobial activity of R. Raetam seed oil. Retama raetam forssk. webb'' is a spontaneous desert shrub, belongs to the fabaceae family with four species include (Retama monosperma, Retama raetam, Retama sphaerocarpa and Retama dasycarpa) mostly distributed in east Mediterranean regions and north Africa. In Libya, commonly found in Fezzan, Tripoli, Ain Zara, Khoms, Tarhuna and Tobruk [11],[12],[13],[14]. The stems, leaves, roots, and flowers were traditionally used. It can be taken by mouth or used externally as compress [15],[16],[17]. However, each part of the plant has a specific medicinal property and thus dedicated its use to treat particular illness. In Libya, some people who practice traditional medicine, recommend using of plant shoots in treatment of diabetes mellitus, sinusitis and antitumor [18]. Also, according to information available from shops that sell aromatic herbs in Tripoli and Zeleiten, seeds taken by mouth to treat worms, while vapor inhalation of shoots used in treatment of Brucellosis. On the other hand, some regions in Libya used it as veterinary drug to heal wounds of sheep mow (personal communication). Generally, Broom brush in North Africa used for treatment of several diseases such as diabetes, hepatitis, jaundice, sore throat, eczema, skin disease, joint pain, rheumatism, inflammation, fever and microbial infection. It is considered as an anthelmintic, emetic, laxative and sedative [19],[20],[21]. In Morocco, crushed leaves and stems mixed with honey and given as an emetic. However, powdered leaves and flowers or their decoction utilized to heal skin diseases. A decoction of leaves given as purgative and anthelmintic [22], [23]. Traditional healers in Saudi Arabia utilized the fruits in treatment of hypertension, diabetes mellitus and hyperlipidemia [24],[25]. In occupied Palestine, plant tea extract was used in treatment of neonatal jaundice, upper branches prepared as vapor bath to treat limb paralysis and boiling of plant roots in water and applying the extract locally on affected area in cases of local paralysis [26], [27]. Furthermore, Tunisian folk medicine, applied its shoots as antidote for snake bites, roots were utilized as antidiarrheal remedy, leaves to reduce arthralgia, back pain and eye infections and curing of renal disease [28], [29]. R. raetam rich with bioactive constituents like flavonoids, terpenoids, quinolizidine alkaloids and fixed oils which are retained biological activities whether toxic or pharmacological actions. Earlier experiments suggested that anti-inflammatory, analgesic and antimicrobial effects were attributed to the presence of valuable secondary products like flavonoids and essential oils that relatively distributed between different plant parts. Previously, flavonoids like daidzein, vicenin-2, naringenin, apigenin, kaempferol, quercetin and kaempferol-7-O-glucoside were detected in the seeds of R. raetam [26], [29].

Material and methods

Plant materials: The Seeds of Retama raetam (forssk.) webb and Berthel were collected from Sobratah area, west region of Libya, in April, 2020. The plant was identified at Herbarium of Department of Botany, Faculty of Science, University of Tripoli, Libya, by two taxonomists: Prof. Mohammed Abuhadra and Dr. Fathi Al-Retib. A voucher specimen for this collection was recorded and kept under code: D-688691.

Chemicals: n-hexane, tween20, tween80, ciprofloxacin disc, phenol.

Instruments and other Materials: Gas chromatography-Mass spectroscopy, sensitive balance, incubator, autoclave, vortex mixer, micropipette, water bath

Culture Media: Muller Hinton agar and sabarouds dextrose agar media

Microbial strains used: Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 25850), Staphylococcus aureus (ATCC 25923) and Candida albicans (ATCC 10213).

Preparation of plant extract: After the collection of plant seeds. Cleaned, shade-dried and powdered by mechanical grind. 500gm of powdered seeds were extracted by Soxhlet apparatus at 60 °C with 1 liter n-hexane for 12 hours. The final net volume of the oil is 35ml (percentage of yield 7%), then the resultant oil stored in amber glass bottle in a cold place [25].

The method of Gas chromatography-mass spectrometry (GC-MS) analysis: The analysis of 1ml of retama raetam seed oil extract was performed using a Thermo Scientific, Trace GC Ultra / ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30m, 0.251mm, 0.1 mm film thickness).For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used, Helium gas was used as the carrier gas at a constant flow rate of 1mL/min. The injector and MS transfer line temperature was set at 280 °C. The oven temperature was

programmed at an initial temperature 40 °C (hold 3 min) to 280 °C as a final temperature at an increasing rate of 5 °C /min (hold 5 min). The quantification of all the identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY library data of the GC/MS system.

Evaluation of antimicrobial activity of retama raetamseed oil against defined pathogens by Agar well diffusion method: The well diffusion method was employed to determine the antimicrobial activities for the R.R seed oil. Different concentrations of extracts, 100%, 50% and 25% were prepared; this is to know the least of the level of concentration that will be required for the culture to grow [28]. About 0.1ml of the standardized 24hour old culture of the tested organisms in Nutrient broth was spread unto sterile prepared Nutrient agar plates and allowed to set. With the aid of a sterile corn borer, hole of 6mm in diameter were bored on the plates, about 0.5ml of each concentrations of the extracts was dispensed into the hole and then allowed to stand for about 15minutes [28]. Also 0.1ml of the prepared ciprofloxacin (standard) and phenol (standard) discs were put into the media. These were then incubated at 37 °C for 24hours. At the end of the period, the zones of inhibition were measured using scientific ruler and the diameters were recorded [28].

Results and discussion

Gas chromatography-mass spectrometry analysis of the seed oil of Retama Raetam.. The GC - MS chromatogram of the seed oil of Retama Raetam showed 47 major peaks indicating the presence of forty seven compounds (Figure1.). The active principles with their peak, retention time (RT), and molecular weight are presented in the (Table 1.) and Reported biological activities of the some identified bioactive compounds in seed oil of Retama Raetam with different research as in (Table 2).

Table 1: Chemical constituents identified in seed oil of Retama Raetam by GC-MS

S.N	RT(min)	Compound Name	Molecular Formula	MW	Peak area
1	5.17	2,7,12,17-tetrabrom-(all-às) cyclotetrathiophen (2,7,12,17-tetrabromcycloocta[1,2-b:4,3-b':5,6-b'':8,7-b''']tetrathiophen	C16H4Br4S4	640	0.47
2	9.99	2-Methylhexadecane	C17H36	240	0.14
3	18.88	2[3,4Bis(dodecyloxy)phenyl]4,4,5,5tetramethyl1,3,2dioxaborolane	C ₃₆ H ₆₅ BO ₄	572	0.26
4	19.42	2,4 - Decadienal	C ₁₀ H ₁₆ O	152	0.45
5	20.28	3-Methoxy-1-pentene	C ₆ H ₁₂ O	100	0.25
6	20.83	Propanoic acid,2methyl,3hydroxy2,4,4trimethylpentyl ester	C ₁₂ H ₂₄ O ₃	216	0.37
7	24.43	Phenol,2,4bis(1,1dimethylethyl)	C ₁₄ H ₂₂ O	206	0.12
8	28.54	Pentadecane	C ₁₅ H ₃₂	212	0.12
9	28.96	Decanoic acid, methylester	C ₁₁ H ₂₂ O ₂	186	0.15
10	31.02	2-Naphthalenol,- 8-amino	C ₁₀ H ₉ NO	159	0.20
11	31.97	1,2Benzenedicarboxylic acid, bis(2methylpropyl)ester	C ₁₆ H ₂₂ O ₄	278	0.47
12	32.12	1,1,1,2tetrafluoro2tridecene	C ₁₃ H ₂₂ F ₄	254	0.11
13	33.03	Dodecanoic acid,3,7,11trimethyl, methylester	C ₁₆ H ₃₂ O ₂	256	19.42
14	33.68	1Octanol	C ₈ H ₁₈ O	130	0.13
15	33.81	3(5Formyl2furyl)2propenal	C ₈ H ₆ O ₃	150	0.55
16	34.28	Octadecane, 1chloro	C ₁₈ H ₃₇ Cl	288	0.18
17	34.44	9- Octadecenoic acid (Z)	C ₁₈ H ₃₄ O ₂	282	0.28
18	34.87	3Undecanol,3ethyl	C ₁₃ H ₂₈ O	200	0.32
19	35.26	NORESERINE	C ₁₄ H ₁₉ N ₃ O ₂	261	5.556
20	36	Phosphonic acid,diocetadecyl ester	C ₃₆ H ₇₅ O ₃ P	586	0.11
21	36.23	9,12Octadecadienoic acid (Z,Z),methyl ester	C ₁₉ H ₃₄ O ₂	294	42.86
22	36.30	9Octadecenoic acid (Z), methyl ester (CAS)	C ₁₉ H ₃₆ O ₂	13.7 7	296
23	36.48	2Hexadecenol,3,7,11,15tetramethyl, [R[R*,R*(E)]](CAS)	C ₂₀ H ₄₀ O	296	0.13
24	37.30	Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	308	0.12
25	38.40	N(2Acetylcylohexylidene)cyclohexylamine	C ₁₄ H ₂₃ NO	221	0.21
26	37.84	Docosane	C ₂₂ H ₄₆	310	0.14
27	37.91	transFarnesol	C ₁₅ H ₂₆ O	222	0.18
28	38.83	4,5dihydro [1,2,4] triazolo[3,4d][1,5]benzoxazepin1(2H)one	C ₁₀ H ₉ N ₃ O	203	0.21
29	39.30	17Octadecynoic acid	C ₁₈ H ₃₂ O ₂	280	0.36
30	39.63	cis11Eicosenoic acid, methyl ester	C ₂₁ H ₄₀ O ₂	324	0.26
31	39.88	3Pinanemethylisothiocyanate	C ₁₂ H ₁₉ NS	209	0.49
32	36.22	Eicosanoic acid, methylester (CAS)	C ₂₁ H ₄₂ O ₂	326	0.41
33	40.52	9-Octadecenoic acid (Z)(CAS)	C ₁₈ H ₃₄ O ₂	282	0.14

34	41.64	2,4bis(áchloroethyl)6,7bis[ámethoxycarbonylethyl]1,3,5trimethylporphyrin	C35H38Cl2N4O4	648	0.17
35	42.09	3(Benzylthio)acrylicacid, methyl ester	C11H12O2S	208	0.19
36	42.39	17-Octadecynoicacid	C18H32O2	280	0.12
37	43.18	Hexadecanoic acid,methyl ester (CAS)	C17H34O2	270	0.15
38	43.56	ethyl3amino2,5dimethyl1,4,6trioxo1,2,5,6tetrahydro4Hpyrrolo[3,4c]pyridine7caeboxylate	C12H13N3O5	279	0.27
39	44.19	17-Pentatriacontene	C35H70	490	0.15
40	46.67	,12- Octadecadienoicacid(Z,Z),2,3dihydroxypropylester	C21H38O4	354	0.13
41	47.45	Squalene	C30H50	410	0.72
42	50.49	çTocopherol	C28H48O2	416	0.68
43	51.54	CHLOROCADMIUM(1R,19R)1,2,2,7,7,12,12HEPTAMETHYL15CYANO19METHOXYCARBONYLCARRINATE	C29H36CdClN5O2	635	0.11
44	52.91	5,10secoCholestan1(10)en3,5dione	C27H44O2	400	0.58
45	53.42	Stigma sterol	C29H48O	412	0.40
46	54.43	1,5Dimethyl6(1,5dimethylhexyl)15,16epoxy18oxatetracyclo[9.6.1.0(2,10).0(5,9)] octdecane13one	C28H46O2	414	1.74
47	54.78	(4Bromophenyl)bis(2,4dibromophenyl)amine	C18H10Br5N	635	0.16

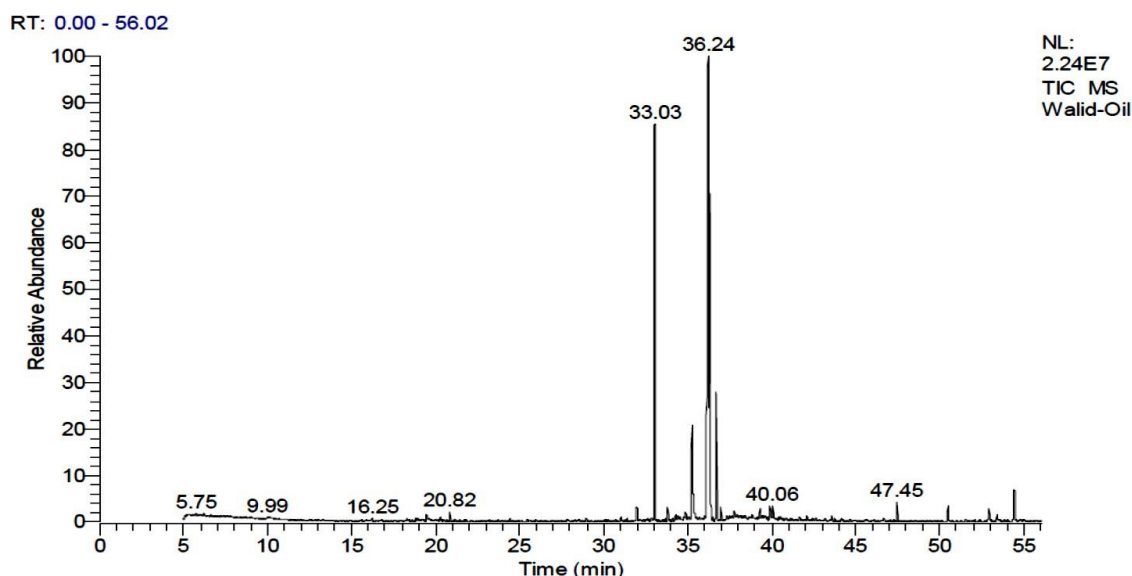


Figure 1: GC-MS spectra of Retama raetam seed oil

Table 2: Reported biological activities of the some identified bioactive compounds in seed oil of Retama Raetam.

Compound	Biological activity
Phenol, 2,4-bis(1,1-dimethylethyl)	Antimicrobial, antioxidant (Salini T.S. et al. 2014).
9-Octadecenoic acid (Z) methyl ester	Antioxidant, anti-cancer (Saada, M et al. 2018).
Squalene	Antioxidant activity (Salini T.S. et al. 2014).
Docosane	Antibacterial (Hamed, A. et al. 2013).
Stigmaterol	Anti-hypercholesterolemia activity (Rahman, M.M. et al. 2014)
Hexadecanoic acid methyl ester	Anti-oxidant, decrease blood cholesterol, anti-inflammatory (Rahman, M.M. et al. 2014).
9,12-Octadecadienoic acid (Z,Z),methyl ester	Antioxidant and antimicrobial activities (Salini T.S. et al. 2014).

Antimicrobial screening of R.R seed oil: The in vitro antimicrobial screening of the seed oil of Retama raetam against Gram-positive (*St.aureus* ATCC 25923), and Gram-negative (*E.coli* ATCC 25922), (*Ps.aureginosa* ATCC25850) bacteria was evaluated in this study and the obtained results revealed activity only against *St.aureusas* shown in (table 3) and no activity recorded against

E.coli and *Ps.aureginosa* as shown in (table 3), and its activity against fungi (*Candida albicans* ATCC 10213) also was evaluated and no activity recorded for different concentrations of the seed oil of Retama raetam (100%, 50%, 25%) as shown in (table 4). Thus indicates that the seed oil of Retama raetam has activity only against *St.aureus*.

Table 3: Antibacterial Activity of seed oil of *Retama raetam* and comparison with standard antibiotic (Ciprofloxacin).

SN	organisms	Zone of inhibition (mm)				
		25mg/ml	50mg/ml	100mg/ml	RR extract	Ciprofloxacin (+CTL)
1	<i>Staphylococcus aureus</i>	-	5 mm	-	-	30 mm
2	<i>Pseudomonas aeruginosa</i>	-	-	-	-	30 mm
3	<i>Escherichia coli</i>	-	-	-	-	30 mm

Table 4: Antifungal activity of seed oil of *Retama raetam* and comparison with control (phenol).

100mg/ml	Candida albicans			RR extract
	50mg/ml	25mg	phenol(+CTL)	
-	-	-	7.5 mm	-

Discussion

GC-MS is the one of the most precise methods to identify various secondary metabolites present in the plant oils. The seed oil of *Retama raetam* was analyzed by GC-MS to detect various compounds present in it. The GC-MS analysis revealed the 47 chemical compounds. (4-Bromophenyl) bis (2,4-dibromophenyl) amine (54.78min) is the highest retention time chemical compound and 2, 7, 12, 17-tetrabrom (allàs) cyclotetrathiophen (5.17 min) as the lowest retention time chemical compound. The most abundant phytochemicals identified in seed oil of *Retama raetam* are 9,12-Octadecadienoic acid (Z,Z), methyl ester (42.86%), Dodecanoic acid, 3,7,11-trimethyl, methyl ester (19.42%) and 9-Octadecenoic acid (Z), methyl ester (13.77%) respectively. The identified compounds were reported in previous studies to have antioxidant, antimicrobial, antioxidant, anticancer and anti-inflammatory activities [26], [29]. The in vitro antimicrobial screening of the seed oil of *Retama raetam* against Gram-positive (*St. aureus* ATCC 25923), and Gram-negative (*E. coli* ATCC 25922), (*Ps. aeruginosa* ATCC 25850) bacteria was evaluated in this study and the obtained results revealed activity only against *St. aureus* as shown in (table 4.3) and no activity recorded against *E. coli* and *Ps. aeruginosa* as shown in (table 4.3), and its activity against fungi (*Candida albicans* ATCC 10213) also was evaluated and no activity recorded for different concentrations of the seed oil of *Retama raetam* (100%, 50%, 25%) as shown in (table 4.4). Thus indicates that the seed oil of *Retama raetam* has activity only against *St. aureus*. The resultant antibacterial exhibited by the seed oil of *Retama raetam* are related to the presence of phytochemicals identified in seed oil of *Retama raetam* which were identified by using GC-MS and reported to have antimicrobial and antibacterial activity in previous studies [26], [29].

Conclusion

The Gas Chromatography-Mass Spectroscopic analysis concluded the presence of bioactive phytochemical compounds in the seed oil of *R. raetam*. It justifies the use of *R. raetam* seeds as an anti-oxidant, anti-inflammatory and antimicrobial agent in herbal medicine.

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