Isolation and Identification of some Active Constituents of Myrtus Communis in Iraq by using HPLC , FT-IR Techniques

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Abstract

The applied research on extract of active constituent from Iraqi myrtus communis leaves using different polarity organic solvents ,then each extract was identify using Fourier transmitting infrared FT-IR and high performance liquid chromatography HPLC which comparing with merck index built in OPUS instrumental of FT-IR(atlas). Some standards were injected in HPLC , therefore we capable to know many organic compounds were present in myrtus should be affected on manner of plant .

الخلاصة

شملت الدراسة استخلاص المواد الفعالة من ورق الاس العراقي ،ومن ثم فـصل المكونـات الكيمياويـة الأساسية باستخدام طرق الفصل الكلاسيكية وباستخدام مذيبات عضوية مختلفة الاستقطابية ،وبعدها عوملـت الأجزاء المفصولة بتقنيتي كروماتوكرافيا السائل ذي الاداء العالي وأشعة تحت الحمراء المنبثقـة لمعرفـة المكونات الموجودة بالنبات وقد تم التعرف على بعض المكونات من خلال معرفة زمن الاحتجـاز لـبعض المركبات ومقارنتها مع مواد قياسية فضلا عن معرفة ترددات المجاميع الفعالة بنوعيها المط والانحناء ضمن مواقع معينة في جهاز الأشعة تحت الحمراء، وقد تم التأكيد على المواد الفعالة التي تؤثر على خاصية النبات من حيث الاستخدام

Introduction

Genus: Myrtus

Species: Myrtus communis L.

Latin name: Myrtus communis

*Synonyms:*common myrtle, 'poivrier corse'

Place of origin: Mediterranean basin, now native to southern Europe and North Africa.

Plant type: an evergreen shrub or a small tree.

Principle Uses **:** Its antiseptic and bactericidal properties make myrtle oil useful in pulmonary ailments[1]. The relatively mild nature of myrtle and its

unobtrusive odor also mean that the oil is suitable to use for children's cough and chest complaints[2][3]. As it is less stimulating than eucalyptus and is slightly sedative, myrtle oil is ideal to vaporize in a room temperature. [4] Patricia Davis recommends its use as a chest rub in 3 % dilutions, while it is also 'a good oil for elderly people both as a treatment and a preventative measure against chest infections'[5] Asthma , bronchitis ,catarrhal conditions ,chronic coughs , tuberculosis. Myrtle's antiseptic and bactericidal properties make it useful for colds and 'flu ,infectious diseases.

Again as antiseptic and bactericidal, myrtle is useful in home use for urinary tract infections and for bladder infections ,infections of the urethra ,antcatarrhal ,antiseptic (urinary, pulmonary) , astringent ,balsamic ,bactericidal ,expectorant ,regulator and slightly sedative.

Gerbeth K et.al[6]. studying the determination of myrtucommulone from Myrtus communis in human and rat plasma by liquid chromatography/tandem mass spectrometry .

The total phenolic content (TPC) of *M. communis* leaves was determined using Folin-Ciocalteu reagent, the mixture was allowed to stand for 2 hrs then the absorbance was measured at 765 nm using spectrophotometer [1] .

The total flavonoids content (TF) of each extract was determined spectrophotometerically using rutin as a reference compound. The absorption of standard rutin solution (0.5 mg/ml) in ethanol was measured under the same conditions. Aliquid-solid extraction and purification procedure (LSE) was developed to identify and quantify polyphenols in the leaf tissue of *Myrtus communis*. Identification and quantitation of individual compounds were performed using HPTLC and HPLC-MS analysis. Leaves of *Myrtus communis* contain small amounts of phenolic acids (caffeic, ellagic and gallic acids) [7][8] ,and quercetin derivatives (quercetin 3-O-galactoside and quercetin 3-O-rhamnoside), whereas catechin derivatives (epigallocatechin, epigallocatechin 3-O-gallate, epicatechin 3-O-gallate) and myricetin derivatives (myricetin 3-O-galactoside, myricetin 3-O-rhamnoside) are present in large amounts. This is the first report on the occurrence of galloyl-derivatives of catechin and gallo-catechin in*Myrtus communis* leaves.[9][10][11]

Experimental

Apparatus :

1- A BRUCKER OPTICS Fourier transmitting infrared (FT-IR)Model ALPH with Attenuated total reflection crystal (ATR)had been established as a standard method for both routine and research applications is the spectral range from 4,000 – 400 cm-1 were used for determination of liquid samples directly with Merck library containing 2000 standard compounds built in memory.

2- A SHIMADZU High performance liquid chromatograph (HPLC) model L 2010A Analyzer with optimum condition :

Type of column :15 Cm X 4.6 mm,5 µM

TM Sulpelcosil LC- 8

Mobile phase : 30/30/40 :Methanol/H_2O/Acetonitrile

Flow rate : 1 ml /min

Temperature : 40 C°

Pressure : 7 mm/Hg

Detector : type U. V at 254 n.m.

Sample size : 15µl

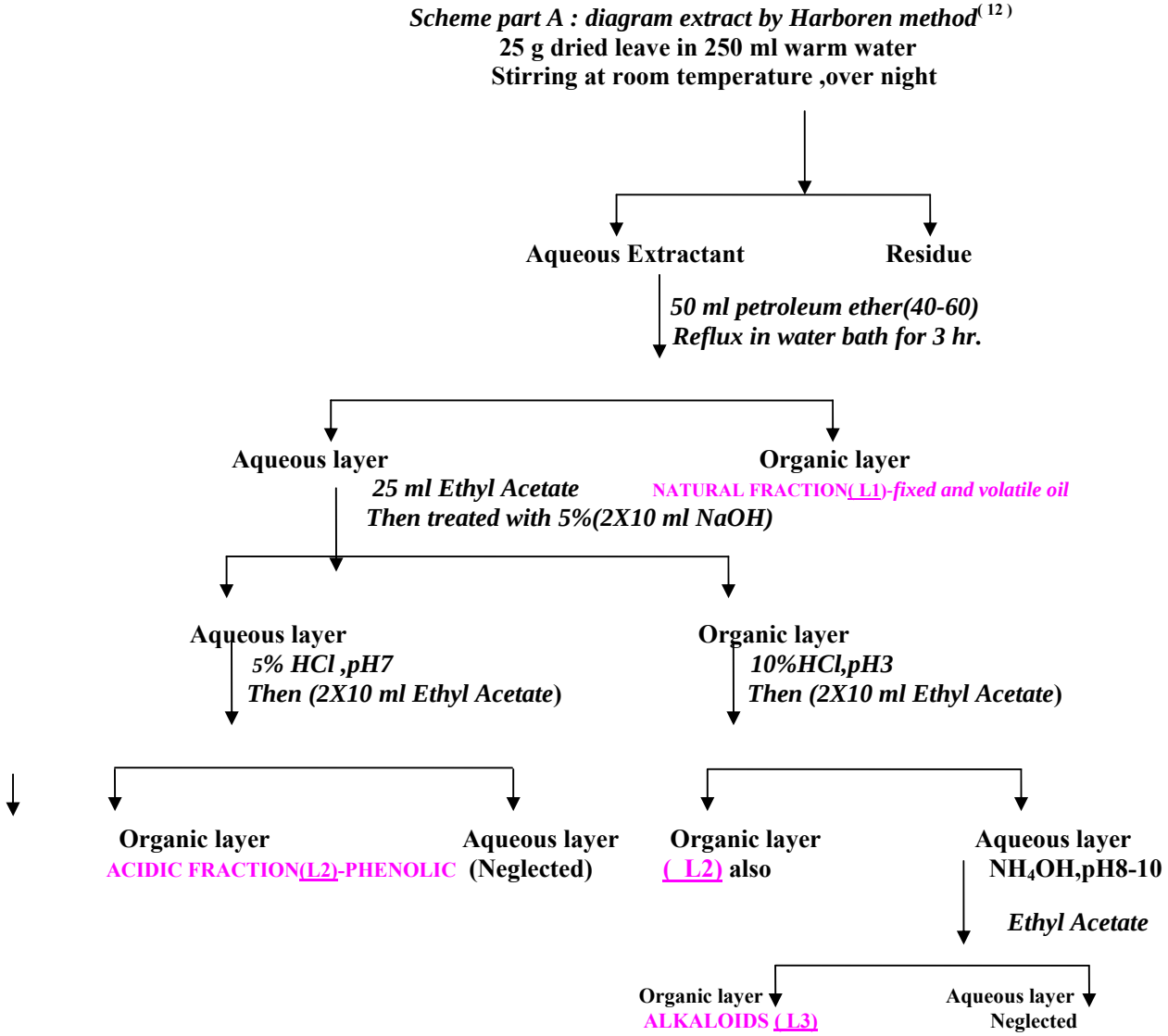
Reagents :

All experiments were performed with analytical reagents grade chemicals:

Petroleum ether(40-60 C),n-hexane , ethanol ,ethyl acetate ,methanol ,acetonitrile ,HCl,NaOH vaseline,glucose,camphor,oleic cid, cortisone , thymol and ibuprofen

Preparation and HPLC/ FTIR analysis of extractants:

Part A:- The air-dried leaves of Myrtus communis (25 g) was extracted with 250 ml of warm distilled water overnight then filtered off to afford aqueous extract, as well as the dried leaves of M. communis (25 g) was extracted with 70% ethanol overnight to afford alcoholic extractant .as shown in the scheme below:



PartB:- Ground air-dried leaves of *Myrtus. communis* (25 gm) were successively extracted with a **series** of solvents in order of increasing polarity :petroleum ether (250ml),n-hexane(250ml),ethanol(250ml)and Water (250ml) at room temperature.The extracts were concentrated by evaporating ,each extract was identified by using FT-IR(ATR diamond) **(5)** and HPLC using column C8 with methanol:H_2O:actonitrile as mobile phase.

Results and Discussion

From Table 1 shows that the kind of active constituents would be extracted by different solvents[13] that which depend on the polarity of solvents. Many fractionations from above schemes A &B were employed by FT-IR instrument, figures 1 and 2 shows the FT-IR spectrum of each extract which the most popular material after comparing with merck index automatically built in soft ware which shown in tables 2 and 3 . Figure 3 and 4 for HPLC spectrum containing the value of retention time for predominate material and comparing with some standards after injection in the column .

Number of transmission signals for IR depend on the number of atom in material as (3N-6) , which N equal the no. of atom ,these transition include stretching and bending .

The most of saturated aliphatic compounds were eluted in petroleum ether fraction see table 4 while it seems that in hexane fraction most aromatic and carbonyl groups. Finally it noted that ethanol and water fractions the rest of phenolic and alkaloid compounds were eluted .

In order to identify the compounds ,some standard were injected in IR spectra and make overlap to know the extract . The same way were applied in HPLC instrument depend on retention time after injection the materials and the practical evidence were gain ,But the quantitative analysis could not be applied due to the huge experiments . In Harborne techniques we see that the oil fraction were extract by petroleum ether as neutral groups (L1),but the aqueous layer contain both phenolic and alkaloid compounds and after addition of alkaline solution the phenolic compounds are hydrolysis to form salt while alkaloid fraction were eluted by ethyl acetate which acidify to be extract ,see the scheme .

Table(1):Type of extractants by different solvents

Solvent	Type of extractants
Petroleum ether, ether, benzene ,chloroform	**Terpenes , oil ,wax ,fatty acid , steroid**
Alcohol , acetone	**Poly vinyl ,sugar(mono & di)**
Water(cold and warm)	**Sugar , starch ,gum , pectin , tannin**
Alkaline hydrolysis	**Acidic phenol , lignin**
Acid hydrolysis	**Simple sugar ,uric acid , lignin**

Table (2): FT-IR and HPLC measurements for active constituents by part A

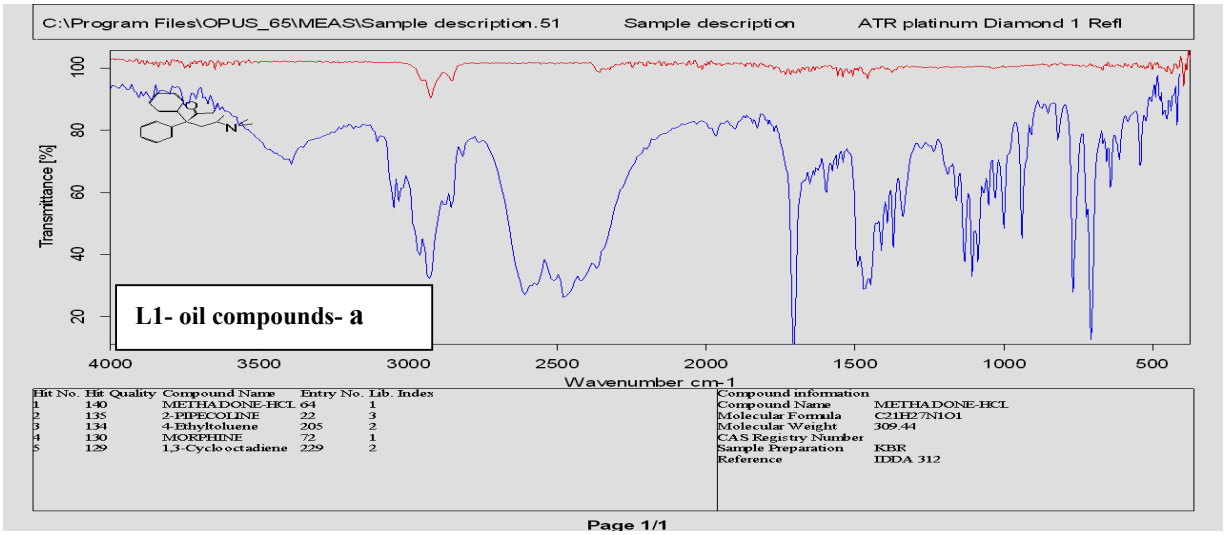
L1 - Oil compounds (expected)	Retention time for HPLC (Min)		L2 – Phenolic compounds (expected)	Retention time for HPLC (Min)		L3 – Alkaloids compounds (expected)
	Standard	Compound in fraction		Standard	Compound in fraction	
Valeric anhydride			Polymethyl methacrylate			Morpholine
Vaseline 8401	**8.5**	**8.9**	2,4-pentandiol			Mowiol 04
Isopropyl myristate			Phthalic acid			N- methyl -2,2-Iminodiethanol
Triethoxy methylsilane			Oleic acid	**2.1**	**1.8**	Isocyanic acid,octadecyl ester
Palmitoyl chloride			1-phenyl ethanol			Thiopheneethanol
Amyl formate			Cortisone	**6.2**	**5.7**	Bis(2-ethylhexyl)phthalate
Camphor	**4.4**	**4.0**	Phenol			
			Thymol	**3.9**	**3.7**	
			D-Glucose	**3.4**	**3.6**	

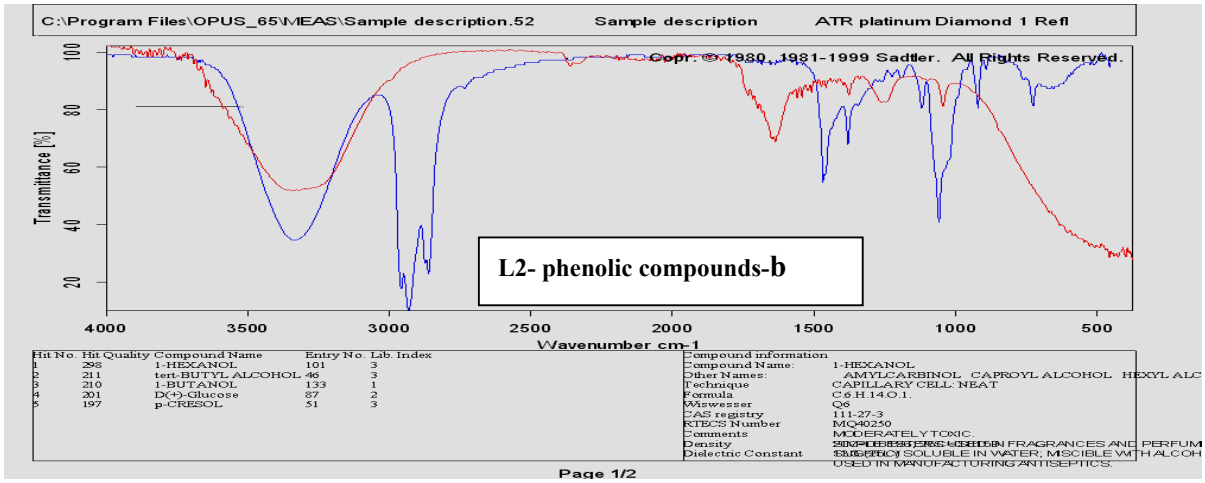
Table (3): FT-IR and HPLC measurements for active constituents by part B

Petroleum ether (sequential) 1/4	n-Hexane (sequential) 2/4			Ethanol (sequential) 3/4			Water(sequential) 4/4		
FT-IR	FT-IR	Retention time for HPLC(min)		FT-IR	Retention time for HPLC(Min)		FT-IR	Retention time for HPLC(Min)	
		Standard	Compound in fraction		Standard	Compound in fraction		St.	Comp.
Methyl benzoate	Methadone-HCl			Polymethylen ethacrylate			Ascorbic acid		
1,1-dichloroethane	3,3-bis[4-hydroxy] 1-[3H]isobenzofuranone			Prednisone			Glucose	**3.4**	**3.5**
Propyl benzoate	Isothiocyanic acid,heptyl ester			DL-Camphor			Anisole		
Valeric anhydride	Methyl acrylate			Lactose			Furfural alcohol		
Chloropromazine	DL-Camphor	**3.2**	**3.1**	Codeine			Pyrrole		
Phthalamide	Prednisone			Ascorbic acid			Furfural		
	Codeine			Cortisone	**6.2**	**5.7**	Adrenaline		
	Thymol	**3.9**	**3.8**	Cocaine					
	Valeric anhydride			Atropine					
	Benzhydrol			Ibuprofen	**2.1**	**2.3**			

Table (4) : Infrared spectroscopic data for eluted fraction.

Solvent	Type of band	Wave number (Cm^{-1})
Petroleum ether	moisture	3356(bw)
	υC-H:CH_3,CH_2,CH Naphthenic group	2927(w)
	υ C=C Aromatic group	1545(w)
	δ C-H:CH_2,CH Naphthenic group	1459(w)
	δ C-H:CH_3 Naphthenic group	1377(w)
	δ C-H Aromatic group	1006(w) 793(s)
Hexane	moisture	3402(b)
	υC-H:CH_3,CH_2,CH Naphthenic group	2932(s)
	C=O group	1734 (s)
	υ C=C Aromatic group	1540(m)
	δ C-H:CH_2,CH Naphthenic group	1454(s)
	δ C-H:CH_3 Naphthenic group	1410(s)
	δ C-H Aromatic group	1148(s) 878(s) 793(s)
Ethanol	moisture	3402(b)
	υC-H:CH_3,CH_2,CH Naphthenic group	2932(s)
	C=O group	1734 (s)
	υ C=C Aromatic group	1540(m)
	δ C-H:CH_2,CH Naphthenic group	1454(s)
	δ C-H:CH_3 Naphthenic group	1410(s)
	δ C-H Aromatic group	1148(s) 878(s) 793(s)





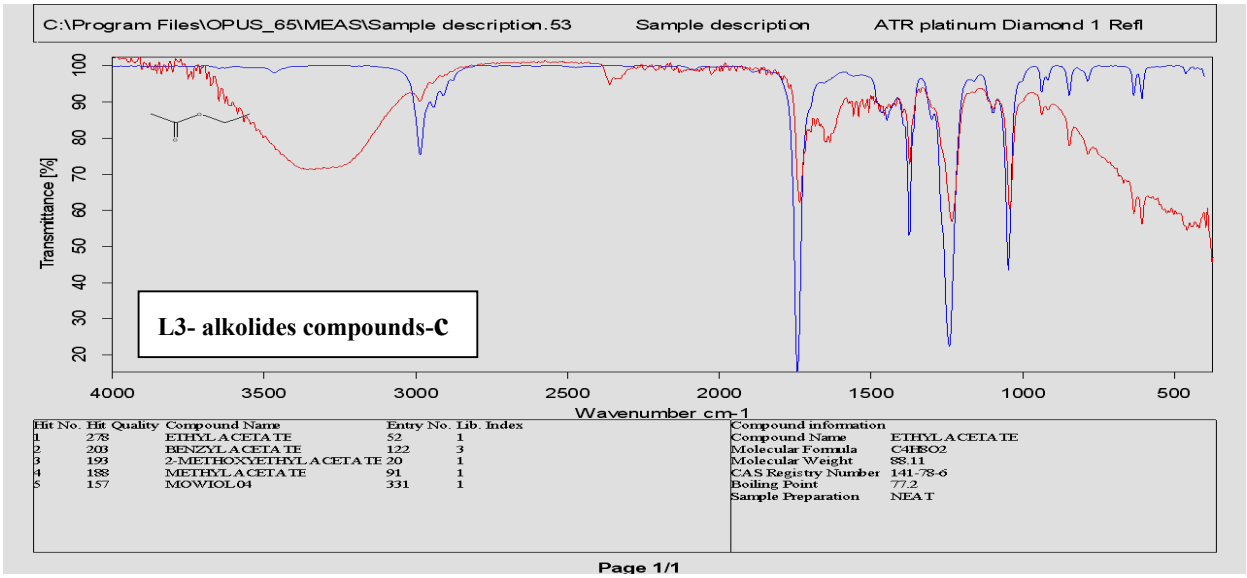
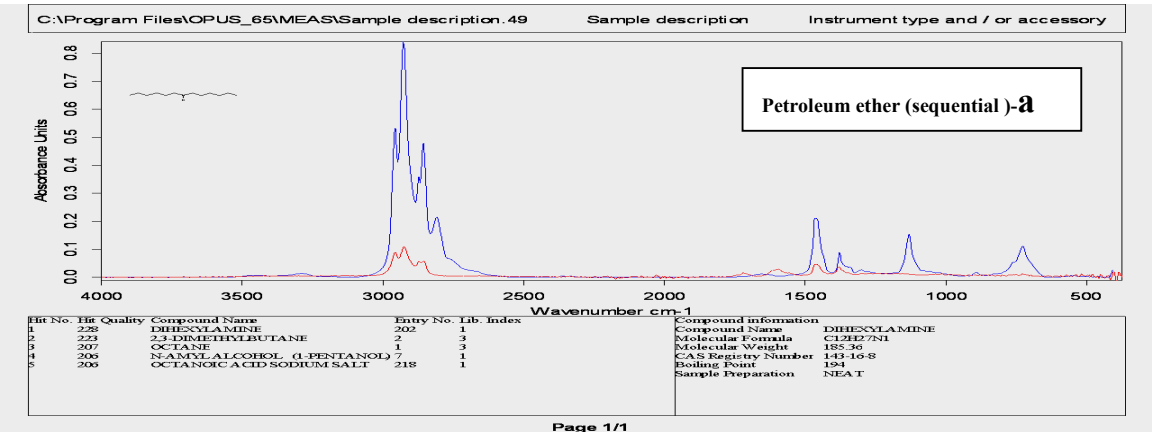
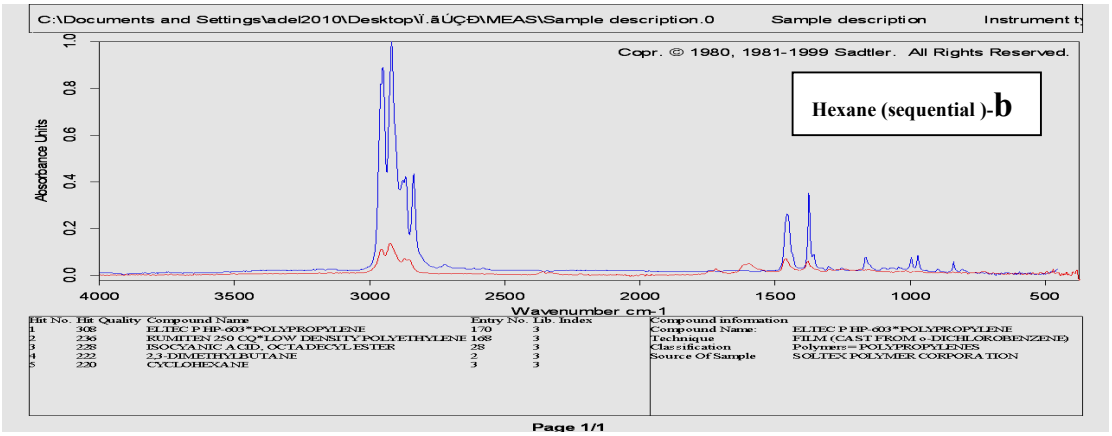
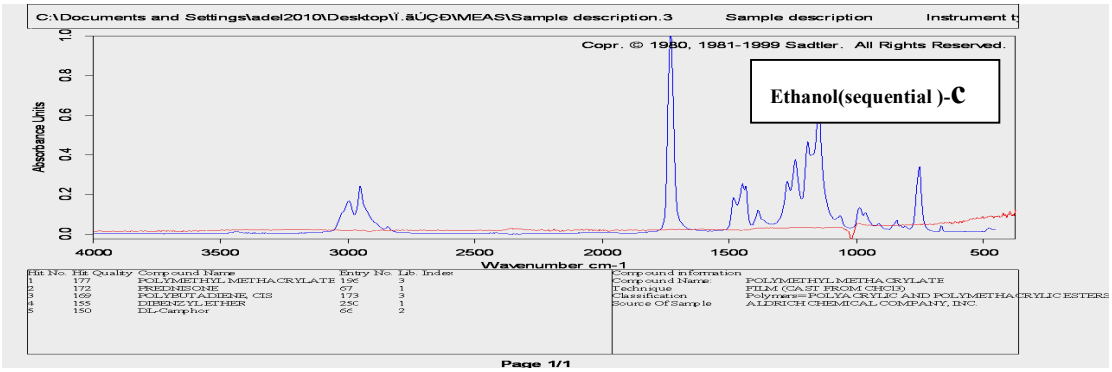
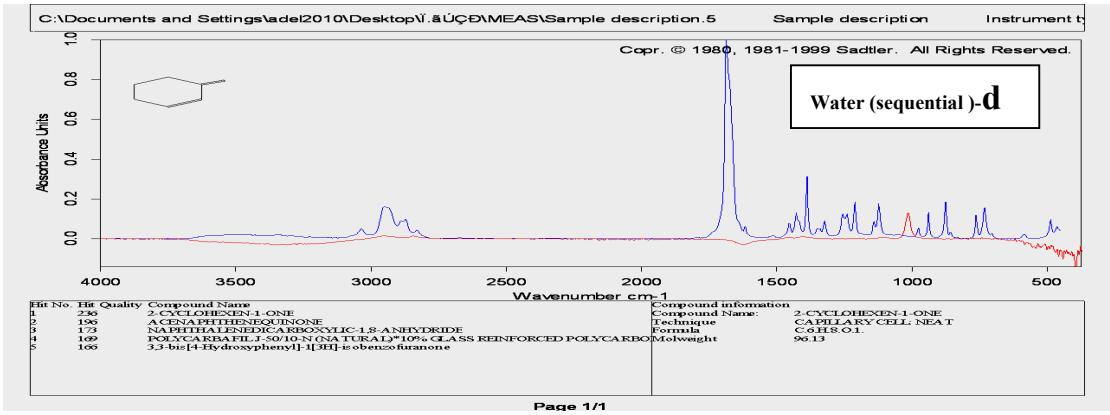


Fig.1(a,b,c):FT-IR spectrum of extractant by scheme part A

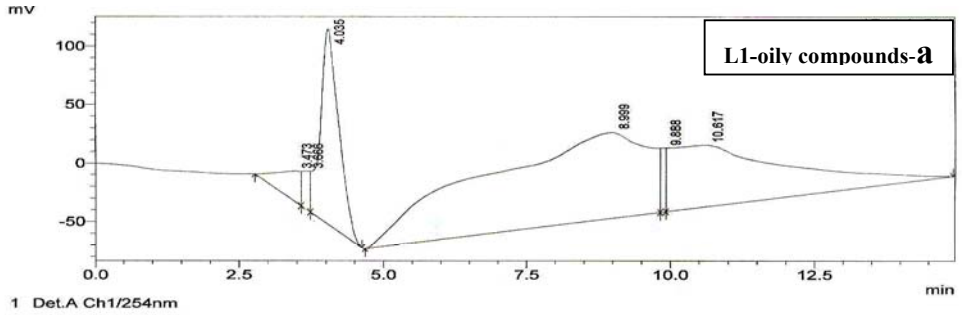








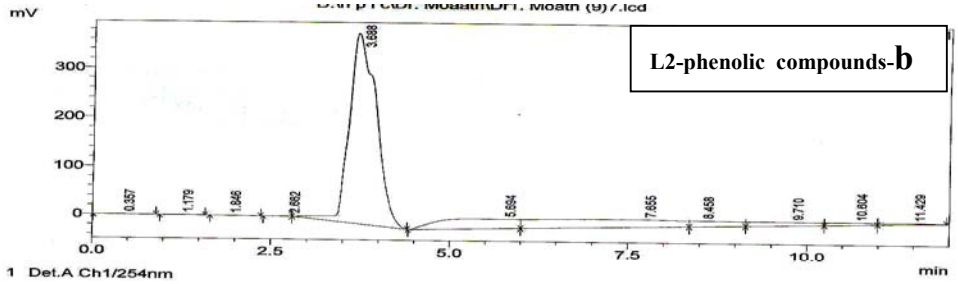
FT-IR spectrum of extractant by scheme part BFig2:(a,b,c,d):



PeakTable

Detector A Ch1 254nm

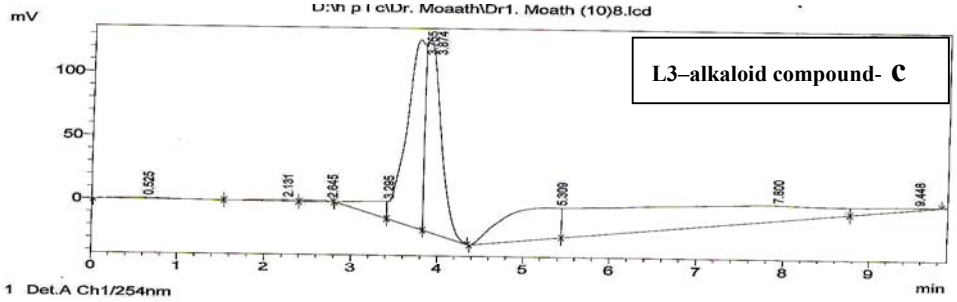
Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.473	712466	26316	2.568	6.451
2	3.666	306263	32798	1.104	8.040
3	4.035	3688626	167136	13.294	40.971
4	8.999	15153558	73682	54.614	18.062
5	9.888	329731	54952	1.188	13.471
6	10.617	7556229	53054	27.233	13.005
Total		27746873	407938	100.000	100.000



PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.357	12767	431	0.082	0.095
2	1.179	5864	257	0.038	0.056
3	1.846	8502	299	0.055	0.066
4	2.662	11915	1084	0.077	0.238
5	3.688	10235511	389859	65.752	85.684
6	5.694	1595792	19741	10.251	4.339
7	7.655	2324869	15718	14.935	3.454
8	8.458	510475	11961	3.279	2.629
9	9.710	513080	7861	3.296	1.728
10	10.604	217793	5006	1.399	1.100
11	11.429	130275	2778	0.837	0.611
Total		15566844	454995	100.000	100.000

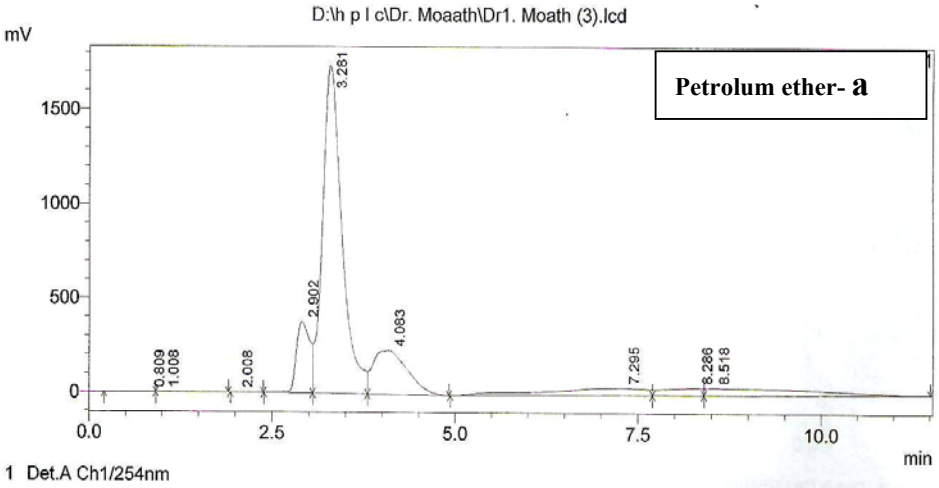


PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.525	35517	822	0.402	0.233
2	2.131	35978	1116	0.407	0.316
3	2.645	22285	1258	0.252	0.357
4	3.295	260226	11461	2.947	3.249
5	3.755	2079982	147990	23.557	41.953
6	3.874	1877335	150169	21.262	42.570
7	5.309	1177184	24255	13.333	6.876
8	7.800	3139653	13220	35.559	3.748
9	9.448	201257	2463	2.279	0.698
Total		8829416	352755	100.000	100.000

Fig3:(a,b,c,):Chromatogram from injection of extractants by HPLC for part A

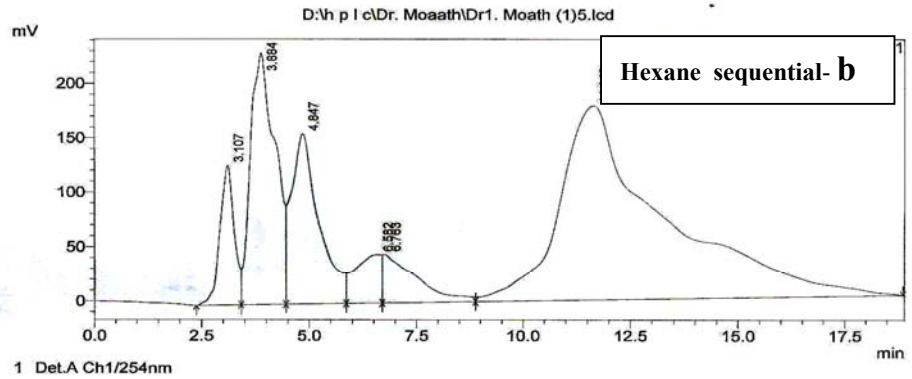


PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.809	15135	576	0.028	0.023
2	1.008	17661	568	0.033	0.023
3	2.008	2413	75	0.005	0.003
4	2.902	4774194	377884	8.908	15.344
5	3.281	32062464	1738240	59.824	70.583
6	4.083	7541604	230591	14.072	9.363
7	7.295	3776582	39384	7.047	1.599
8	8.286	1461257	37930	2.727	1.540
9	8.518	3942897	37446	7.357	1.521
Total		53594208	2462693	100.000	100.000

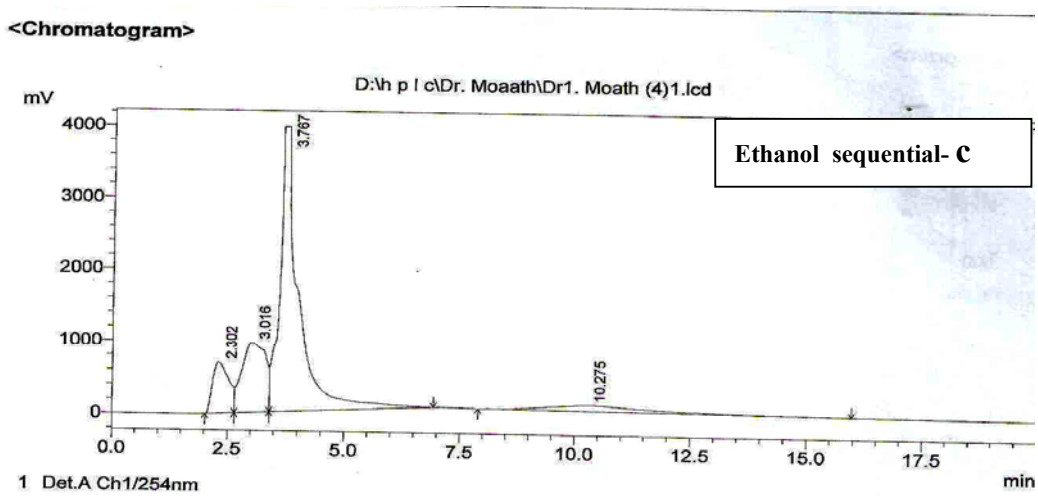
<Chromatogram>



PeakTable

Detector A Ch1 254nm

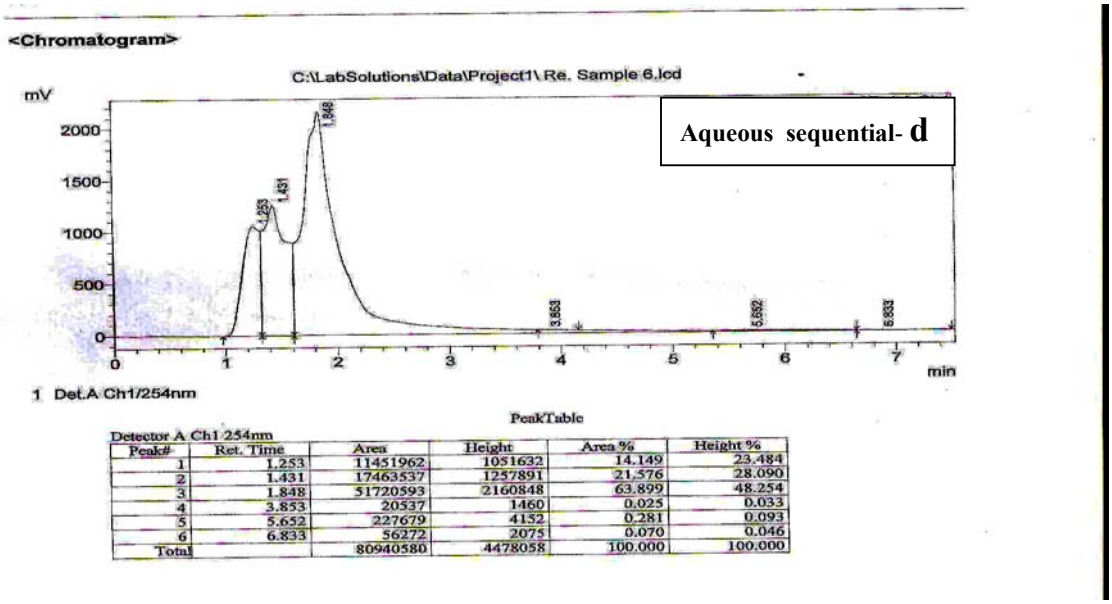
Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.107	3006041	128411	5.587	16.374
2	3.884	9237929	231229	17.170	29.485
3	4.847	7019730	156610	13.047	19.970
4	6.582	1906868	44691	3.544	5.699
5	6.763	2449577	44765	4.553	5.708
6	11.646	30181750	178529	56.098	22.765
Total		53801895	784235	100.000	100.000



PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.302	16724827	693517	9.641	12.203
2	3.016	33676195	956484	19.413	16.831
3	3.767	110793408	3952980	63.868	69.558
4	10.275	12278130	80004	7.078	1.408
Total		173472560	5682986	100.000	100.000



PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.253	11451962	1051632	14.149	23.484
2	1.431	17463537	1257891	21.576	28.090
3	1.848	51720593	2160848	63.899	48.254
4	3.853	20537	1460	0.025	0.033
5	5.652	227679	4152	0.281	0.093
6	6.833	56272	2075	0.070	0.046
Total		80940580	4478058	100.000	100.000

Fig4:(a,b,c,d):Chromatogram from injection of extractants by HPLC for part B

CONCLUSION

We conclude that the myrtus leaves contain terpenes ,alkaloids and phenolic compounds which affected on the manner of plant, that is the most important to analyzed of plant by instrumental techniques ,but have many components which impossible to it has identify and need tedious procedures to characterized ,therefore we were capable to know some component available.

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