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Extraction and Characterization of some active Organic compounds from Breckland thyme plant.

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ABSTRACT

This study focused on diagnosis of volatile oil extracted from Breckland thyme leaves plant by aqueous solvent by Reflux method. Thin layer chromatography was used to separate some active organic compounds from Breckland thyme leaves oil. (Toluene:Ethyl acetate) (5:95) used in Thin layer chromatography as eluent. Results showed 5 spots, 4 spots were characterized by different spectroscopic methods qualitative and quantitive UV–VIS.,IR, 1H-NMR Spectra beside other analytical method gas chromatography (G.C). Results indicate that the 14 compound in A very small percentage four of that following compounds, Carvacrol, p-Cymene, Camphor and thymol are present in the following percent, 23.7, 10.44, 0.945, 19.77 respectively in Breckland thyme oil leaves and unknown compounds in Breckland thyme plant.

Introduction:-

The Treatment with medicinal plants continued till the 19th century when chemistry science started its progress and then chemists started to extract active compounds from different plants and trees [1].

Thyme has a long history of use in natural medicine in connection with chest and respiratory problems including coughs, bronchitis and chest congestion [2].

Researchers have pinpointed some of the components in Breckland thyme that bring about its healing effects [3].

For thousands of years, herbs and species have been used to help preserve foods [4] protect them from microbial contamination, research shows that both thyme and basil contain constituents that can both prevent contamination and decontamination previously contamination foods[5].

Oil of thyme is derived from thyme, also known as thymus vulgaris [6]. The perennial herb, a member of the main family is used in aromatherapy, cooking, potpourri, mouth washes and elixirs, as well as, added to ointments [7]. Thyme has also a number of medical properties, which is due to the herb's essential oil [8]. The medical properties of Breckland thyme oils (which are extracted through steam distillation of fresh flowers and leaves) are due to their component Acne, Anticancer, Antispasmodic, Antirheumatic, Antiseptic, Bactericidal, tonic, cordial, carminative, insecticide stimulant, yeast killer and others[9]. Al-sadec. et al., study showed that a combination of vegetable oil extracted thyme and lemongrass with the effectiveness of inhibitory less than the use of oil extracted individually against Candida species[10]. omran. et al., studied the effectiveness of oily extracts of plants (thymus, lemon, pennyroyal) against candida spp Abstract vegetation gave highly effective against Candida species higher than the oily extract of vegetarian lemon and pennyroyal[11]. The aim of this research was focused on isolation and characterization active organic compounds in Breckland thyme.

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Experimental:-

Dry plant thyme was obtained from market, it is identified by Education College for pure science, university of Anbar, it was air dried and packed in plastic containers.

Extraction of Breckland thyme oil:-

In 1 litter a round bottomed flask, 200 cm^2 of distilled water was added to 40 gm of plant. The extraction process was carried out for 3.5 hours, and then the volatile oil was separated from the aqueous phase with ethers and then dried with Na_2SO_4 anhydrous. Filtrate, evaporate in water bath at 40 ºC the oils obtained were stored in dark battle.

Thin layer chromatography:- From different solvents, we choice the mixture of ethyl acetate and toluene in percent 5%and 95% respectively, as mobile phase while suitable plastic paper coated with silica gel was chosen as a stationary phase. The chromatogram shows five zones, each zone have been scratched, isolate and dissolve in ether, than filtration. Removal of the solvent gave the desired compounds. The identification process depends on UV-VIS. spectra, IR and H-NMR spectra and the value of R_f when compared with standard. Compound under the same condition four zones were identified, while two zones are unknown. Gas chromatography also used to identify all components of oil [12].

UV-VIS. Spectra:- UV-VIS. spectra were recorded using Schimadzo UV-VIS spectrophotometer 715 over the range 200-800 nm, by using DCM as solvent, to analyses the compounds that has been isolated in this study from the preparative TLC.

IR spectra:- IR spectra were recorded in University of Erbil College of science using Schimadzo spectrophotometer over the range 600-4000 cm^{-1} to analyses the compounds that has been isolated.

^{1}H-NMR spectra:- all ^{1}H-NMR spectrum were recorded in Irbaid University –Jordan, using $CDCL_3$ as solvent.

Gas chromatography:- Gas chromatography analysis was carried out in resources directorate Almaiah –Arbial by using a GC apparatus, using GC 717 from Schimadzo equipped with a flam ionization detector.

Results and discussion:-

Fig(1)appear the T.L.C test of Breckland thyme oil, from this chromatogram, one could observe six zone ,four zones were characterized when compared with standard material, the results of these comparisons were explained in Table(1) and figure (1).

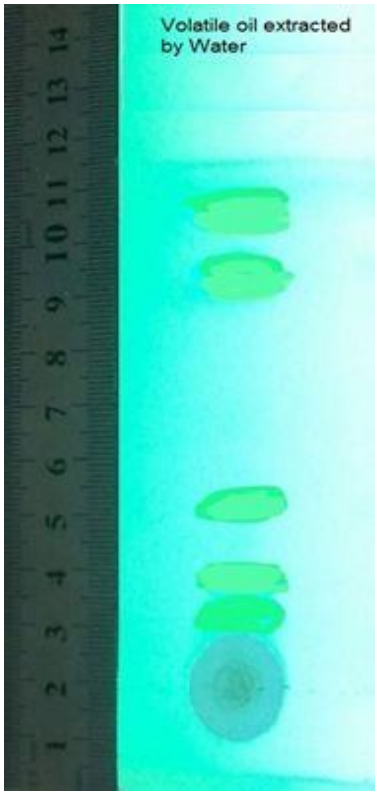


Fig 1:- TLC chromatogram.

Tabel 1:- Retention factor (Rf) of identification compound for volatile oil.

Compound	Flow rate R_f
p-Cymene	0.92
Thymol	0.87
Carvacrol	0.60
Unknown	0.31
Camphor	0.20

*ethyl acetate: Toluene 5: 95 % as mobile phase

To identified each component of this chromatogram by spectroscopic methods. Each zone was crushed carefully isolated and dissolved in diethyl ether, which on filtration and removal of solvent gave the desired compounds.

Uv.vis of compounds shown (P-cymene) showed λmax at 237 nm this is due to the π-π* transitions, λ 220 nm and λ 205 nm. Thymol compound showed λ at 408 nm, λmax 238 nm this is due to π-π* and λ 210 nm. carvacrol compound showed λ at 374 nm, λ 339 nm. This is due to n-π* and λmax 251 nm due to π-π*. Saturated ketone camphor shows λmax at 237 nm, λ 229 nm due to n-π* transition and λ 203nm due to π-π*. α-pinene shows λ at 374nm, λmax 255 nm. This is due to π

-π* transitions and λ 233 nm. While the remaining peaks caused effect on the solvent Diethyl Ether.some absorbents in figure (2) and Table (2) .

FT.IR spectrum of carvacrol in revealed a band in the region 3267cm^{-1} due to stretching vibration of the phenolic O-H group. The presence of (OH) absorption band was a clear proof and a good indication of the success of preparation reaction. 2926-2856 cm^{-1} is due to C-H aromatic ring stretching, 1521-1460-1458 cm^{-1} is due to C=C aromatic ring stretching band, 1458-1241 cm^{-1} is due to the O-H bending vibration, the peak at 1395 cm^{-1} refers to the isopropyl group, a strong band at 1251 cm^{-1} is due to C-O stretching, and at 802 cm^{-1} is due to aromatic C-H bending. The most prominent and informative bands in the spectrum of the aromatic hydrocarbon p-cymene [1-isopropyl-4-methylbenzene] appeared in 3080 cm^{-1} is due to aromatic C-H stretching band, 2982-2858 cm^{-1} is due to C-H stretching of alkane, 1520 cm^{-1} is due to C=C aromatic ring stretching, 1388 cm^{-1} is due to CH_3 symmetrical bending. 1055 cm^{-1} for in-plane bending C-H band, and 813 cm^{-1} at a low frequency range due to C-H out-of-plane bending. The FT.IR spectrum of camphor in revealed a clear, broad stretching band 2950 cm^{-1} , 1743 cm^{-1} is due to a carbonyl group stretching band, 1384 cm^{-1} is due to CH_3 stretching band and 767 cm^{-1} is due to C-H bending. FT.IR spectrum for Thymol in exhibits a broad band which appeared at 3485cm^{-1} assigned to the stretching vibration of (OH) phenolic group. The region 3010 cm^{-1} is due to stretching of C-H aromatic, 2970-2864 cm^{-1} is due to stretching of alkane, 1448 cm^{-1} is due to the C=C aromatic ring stretching. In the region 1350 cm^{-1} is due to the O-H in plane bending vibration, 1294 cm^{-1} is due to isopropyl group region, a strong band 1383 cm^{-1} is due to C-O stretching in phenol and 794 cm^{-1} out-of-plane is due to the aromatic C-H bending in Table(3)..

The H-MNR spectra of compounds were shown in Table (4) give the chemical shift of each proton in these compounds. Table(4) gives the maximum absorption bands (λ_{max} in nm) of compounds. show the chromatogram of Breckland thyme oil which was measured by gas chromatogram (GC), the components of this oil were identified by comparing their relative retention times with those of authentic samples [13] [14].

Thymol give five signals: 1-signal at δ:1.2 ppm doublet is due to CH_3 containing one hydrogen atom, 2-signal at δ:2.25 ppm singlet is due to CH_3 correlation with carbon atom not containing hydrogen, 3-signal at δ:3.17 ppm multiplet is due to CH correlation with the two carbon atom containing six hydrogen atom, 4-signal at δ:4.7 ppm singlet is due to hydroxyl group OH, 5-signal at δ:6.80-7.10 ppm multiplet showed two carbon contain one hydrogen atom aromatic.

Carvacrol give five signals:- 1-signal at δ:1.3 ppm doublet due to CH_3 correlation with the carbon atom containing one hydrogen atom, 2-signal at δ:2.25 ppm singlet is due to CH_3 correlation with carbon atom, not containing hydrogen, 3-signal at δ:2.9 ppm multiplet CH correlation with two carbon atom containing six hydrogen atom, 4-signal at δ:5.9 ppm singlet due to hydroxyl group OH, 5-signal at δ:7 ppm multiplet is due to two carbon atom aromatic.

P-cymene give the following signals: 1-signal at δ:1.2 ppm doublet due to CH_3 correlation with carbon atom containing one hydrogen atom, 2-signal at δ:2.3 ppm singlet is due to CH_3 correlation with the carbon atom, not containing hydrogen, 3-signal at δ:2.9 ppm multiplet due to CH correlation with two carbon atom, containing six hydrogen atom, 4-signal at δ:7.2 ppm multiplet showed two carbon aromatic.

Camphor give the following signals: 1-signal at δ:0.7 ppm singlet due to CH_3 correlation with the carbon atom, not containing hydrogen, 2-signal at δ:0.8 ppm Singlet due to CH_3 correlation with the carbon atom, not containing hydrogen, 3-signal at δ:1.0 ppm triplet due to CH_2 correlation with CH and carbonyl, 4-signal at δ:2.3 ppm quartet due to CH_2 correlation with two carbon atom contain three hydrogen atom, 5-signal at δ:5 ppm doublet CH_2 due to correlation with carbon atom contain hydrogen atom. 6- signal at δ:5.2 ppm multiplet due to CH correlation with two carbon atom contain four hydrogen atom.

Gas chromatography The density and percentage of weight for active organic compound in Breckland thyme oil were found in figure (3) as follows: (α-Pinene) is 39.85*103 g.cm-3 and the percentage is 19.92w/w %, (p-cymene) is 20.9*103 g.cm-3 and the percentage 10.44 w/w %, (Camphor) is 1.9*103 g.cm-3 and the percentage is 0.945 w/w %, (Thymol) is 39.55*103 g.cm-3 and the percentage is 19.77w/w %. Carvacrol is

47.4*103 g.cm-3 and the percentage is 23.7 w/w %. While the volume that is used in measurement is (75*10-3 cm3) in figure (3).

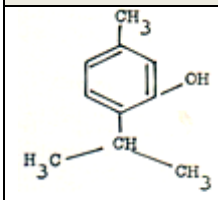
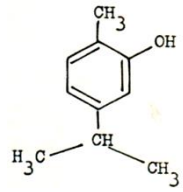
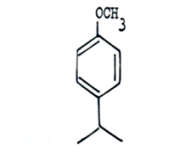
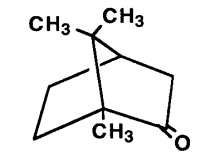
Table 2:- UV-VIS. Spectral data of compounds

Compound	Absorption maxima λmax (nm)
Thymol	210,238,408
Carvacrol	374,339,251
p-Cymene	205,220.237
Camphor	203,229,237

Table 3:- Some characteristic bands of compounds

Compound	Name	Assignment cm^{-1}				
		OH	CH Str.	C=C Str.	OH bending	Isopropyl group
1	Thymol	3485 broad	2962-2870	1570-1381	1350	1285
2	Carvacrol	3267	2926-2856	1521,1460,1458	1458-1241	1395
3	Camphor	-	2950	-	-	1390-1375
4	p-Cymene	CH aromatic str. 3080	2982-2858	1539		
		C-O str. In phenol		CH aromatic bending		C=O
		1246 1251 -		792 802 750		1750

Table 4 ^{1}H-MNR data of active organic compounds

Compounds	Chemical shifts:PPM				
	A	B	C	D	Aromatic protons
 Thymol	1.2 (d)	2.25 (s)	3.17 (m)	4.7 (s)	d 6.80-710
 Carvacrol	1.3 (d)	2.25 (s)	2.9 (m)	5.9 (s)	7.0(m)
 p-Cymene	1.2 (d)	2.3 (s)	2.9 (m)		7.2(m)
 Camphor	0.7 (S)	0.8 (S)	1.0 (S)	Other (2.3)	Multiplet

S: Single, d: doublet, m: multiplet

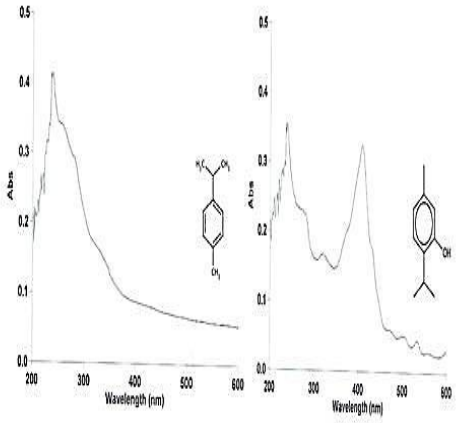


Fig (A) of compound P-cymene Fig (B) of compound Thymol

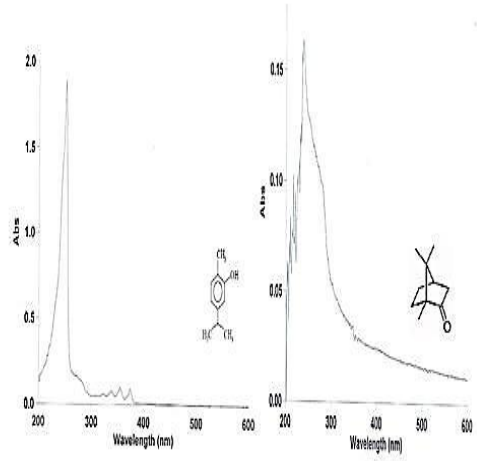


Fig (C) of compound Carvacrol Fig (D) of compound camphor

Fig (2) UV- Vis spectrum of active organic compounds in volatile oil by using Diethyl Ether solvent at room temperature

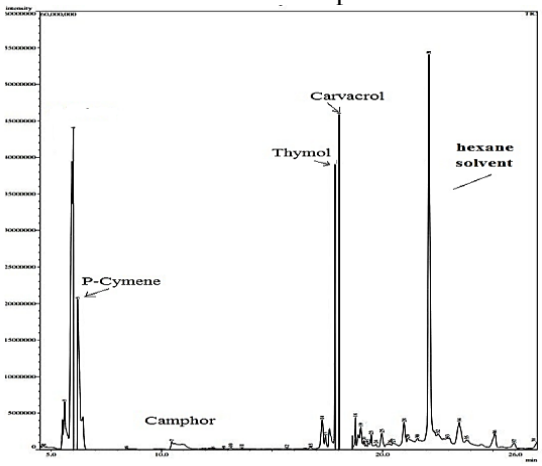


Fig (3) Gas chromatography of active organic compound in the volatile oil of Breckland thyme by using solvent Hexane

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استخلاص وتشخيص بعض المركبات العضوية الفعالة من نبات الزعتر البري

قتيبه فاضل العاني* فؤاد حسين # صبري محمد المرسومي

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الخلاصة :-

ركزت هذه الدراسة على تشخيص الزيت الطيار المستخرج من اوراق نبات الزعتر البري بواسطة المذيب المائي في طريقة التصعيد. استخدمت كروماتوغرافيا الطبقة الرقيقة لفصل بعض المركبات العضوية الفعالة من أوراق نبات الزعتر البري. استخدمت كروماتوغرافيا الطبقة الرقيقة (التولوين: خلات الإيثيل) بنسبة (5:95). وأظهرت النتائج 5 بقع، وتم تشخيص 4 بقع منها بطرق طيفية مختلفة نوعية وكمية وهي الأشعة فوق البنفسجية المرئية، الأشعة تحت الحمراء، طيف الرنين النووي المغناطيسي للهايدروجين بجانب التحليل بواسطة كروماتوغرافيا الغاز .(G.C) تشير النتائج إلى أن 14 مركب تم الحصول عليها في نسب صغيرة جدا أربعة من المركبات التالية، كارفاكرول، بارا سيمين، الكافور والثيمول موجودة في النسب التالية، 23.7، 10.44، 0.945، 19.77 على التوالي في زيت الزعتر البري ومركبات اخرى غير معروفة في نبات الزعتر البري.