تحـــديد المركبـات الكيميائية الموجـــودة فـــي نبات الكـــراوية بواســطة تقنية GC / MC ودراســـة تأثيراتها البيولوجيـــة

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الملخص:

تـــم تجفيف أجـــزاء النبات *Carum carvi* (200.0 جم) في الظل عند درجة حـــرارة الغرفة 22 درجة مئوية وطحنها جيدًا، تم استخلاصها بكميات متساوية من Pet.Ether / CHCl3 / MeOH (3 × 1 لتر) في درجة حرارة الغرفة لمدة أسبوعين بعد ذلك، أدّى تبخير المذيبات تحت ضغط منخفض بواسطة المبخر الدوراني إلى الحصول على 95.56 جم من المستخلص الخام لنبات الكراوية , فقط 71.67 جم من مستخلص Pet.Ether / CHCl3 / MeOH لنبات *Carum carvi* المذابة جيدًا في مذيب مائي (H_2O) ، والتي تم الحصول عليها بعد استخلاصها جيدًا ثلاثة مرات عن طريق الفصل بالقمع باستخدام ثلاثة مذيبات عضوية مختلفة (Pet. Ether و Chloroform و Ethyl Acetate) للحصول على ثلاثة كسور من النبات

- ❖ جزء البترولیم ایثیر (أ): 20.00 جم
- ❖ جزء الكلوروفورم (ب): 33.00 جم
- ❖ جزء خلات الإيثيل (ج): 18.67 جم

تم تحديد مســتخلص Pet.Ether / CHCl3 / MeOH لنبات *Carum carv* بتقنيـة GC / MS ، تم التعرف على **25** مركبًـا فقط من خلالهم , و تم الإبلاغ عن مماثل من قبل NIST.

تم دراســـــــــــــة الأنشطة المضادة للميكروبات لمستخلصات (البترولیم إيثر، الكلوروفورم، الإيثيل أسـيتات) من نبات الكراوية. وتم فصـل واختبار المسـتخلص الكلي. تم إذابـة كل مسـتخلص بعد تبخيره وتجفيفه في ثنـائي ميثيل سـلفوكسـيد (DMSO) لتكوين تركيزات 1 و 10 و 25 و 50 مجم / مل، ثم تم فحصـها بحثًا عن وجود أنشـطة مضـادة للميكروبات باسـتخدام خمسـة بكتيريا ممرضـة منها الايجابية لصبغة جرام مثل *Staphylococcus aureus* و *.cereus B* والسالبة لصبغة جرام مثـل *Escherichia coli*، *Pseudomonas aeruginosae* و *Klebsiella pneumonia*. وأيضـا تم فحص جميع المسـتخلصـات النباتية كمضـادات للفطريات باسـتخدام أنواع مختلفة من الفطريات المسـببة للأمراض: *Candida albicans* ،

Penicillium notatum، *Alternaria alternata*، *Aspergillus niger* و *Fusarium oxysporium- pisi*.

لوحـــــــظ أن الأنشطة المضادة للبكتيريا والفطريات تزداد خطيًا مع زيادة تركيز المستخلصات (مجم / مل). وجد أن الكلـــــــوروفورم يثبط نمو جميع الكائنات الحية الدقيقة وأظهر التأثير مدى واسـع ضـد كل من البكتيريا موجبة الجرام (*S. aureus* ؛ 12 مم و *B. cereus* ؛ 15 مم) والبكتيريا ســالبة الجرام (*P. aeruginosa* ؛ 18 مم). ومن نـاحية أخرى مســتخلص الأثير البترولي ومســتخلص ETOAC وجميع مستخلصات *S. aromaticum* خالية تمامًا من أي نشاط ضد بكتريا الالتهاب الرئوي *K. pneumonia*. من ناحية أخرى . لم تظـــهر من مستخلصات *Carum carvi* أي نشـــاط ملحوظ ضـــد جميع الفطريات المختبرة بجميع التركيزات. أظهر التحليل الإحصـائي أن تأثير نوع المسـتخلص النباتى كان أقوى (بنسـبة F أعلى) من التركيز لجميع الأنواع البكتيرية والفطرية. بينما ، في *S. aromaticum* ؛ كان تأثير التركيز أقوى (بنسبة F أعلى) من نـــوع المستخلص لجميع الأنواع البكتيرية .

تـــم فحص القدرة الكلية المضادة للأكسدة للمستخلصات المختلفة بتركيزات مختلفة من نبات الكراوية و لوحظ أنه مع زيادة تركيز أي من هذه المسـتخلصـات، زاد نشـاط مضادات الأكسدة- أيضًا- .

Determine the chemical composition present in caraway by GC/MC technique
and study their biological activity effects

INTRODUCTION

Role and biosynthesis of Natural products (plant secondary metabolites)

Plants are a valuable source of a wide range of natural products (secondary metabolites) [1]. Secondary metabolites are chemicals or compounds present in plants that are not involved in the primary biochemical processes of plant growth and reproduction. Plant secondary metabolites however are known to play a crucial role in plant protection from insect predation or grazing by herbivores and adaptation of plants to their environment [2, 3].

The secondary metabolites are biosynthesized from primary metabolites by several pathways such as shikimic acid, tricarboxylic acid

cycle and malonic acid pathways [3]. These plant materials or compounds fall into the category of saponins, tannins, lignins, volatile oils, , alkaloids to mention a few [2]. Discoveries in plant secondary metabolites state that different biosynthetic pathways are responsible for diversity of classes or/and groups of plant components for example. 29,000 terpenes resulting from isopentenyl diphosphate (IPP), 12,000 alkaloids from (amino acid) and 8,000 phenolics produced by shikimate or acetate malonate pathway. IPP modification pathway leads to a series of monoterpene compounds, mainly, (-) limonene, (-) isopiperitenone, (+) isomenthone, (-) –menthol [4]

It has been confirmed that the above compounds belong to the monoterpene groups that play important protective role in the plant kingdom. Limonene and menthol are two well - known monoterpenes, which serve as defenses against insects and other organisms feeding on plants [4]

The biosynthetic pathways of different terpene groups from plants. During this process, IPP and DMAPP are converted to give diverse groups of terpenes that play important role in plant protection [4, 5]. These components have complex and unique structures, resulting from both biotic and abiotic stress enhanced conditions, are stored in specific cells and/or organs of the plant, and often accumulate in vacuoles [5]

Caraway (*Carum carvi*).

Caraway also known as meridian fennel and Persian cumin (*Carum carvi*), is a biennial plant in the family Apiaceous, native to western Asia, Europe, and North Africa

Caraway is normally a biennial and multi branched herb, 35-70 cm in height, with narrow finely grooved leafy stems. It produces a deep taproot and a rosette of dark green, finely cut, feathery leaves. It has a high vernalisation requirement to initiate the production of flowering stems in the and 2-3 mm across, the outer ones larger than the inner ones. They open from late April onwards and are succeeded by fruits which are 3-6 mm long, and light brown, ripening from early June and July [6].

Names.

The etymology of caraway is complex and poorly understood. Caraway has been called by many names in different regions, with names deriving from the Latin *cuminum* (cumin), the Greek *karon* (again, cumin),

which was adapted into Latin as *carum* (now meaning caraway), and the Sanskrit *karavi*, sometimes translated as "caraway", but other times understood to mean "fennel".

Uses.

The fruits, usually used whole, have a pungent, anise-like flavor and aroma that comes from essential oils, mostly carvone, limonene, and anethole. Caraway is used as a spice in breads, especially rye bread.[7] In the United States, the most common use of caraway is whole as an addition to rye bread – often called *seeded rye* or *Jewish rye* bread, where the recipe itself owes to East Slavic coriander and caraway flavored bread (see Borodinsky bread). Caraway fruits are frequently used in Irish soda bread, along with raisins and currants. Caraway is also used in desserts, liquors, casseroles, and other foods. Its leaves can be added to salads, stews, and soups, and are sometimes consumed as herbs, either raw, dried, or cooked, similar to parsley. The root is consumed as a winter root vegetable in some places, similar to parsnips.[7]

Caraway fruits are found in diverse European cuisines and dishes, for example sauerkraut, and caraway seed cake. In Austrian cuisine, it is used to season beef and, in German cuisine, pork. In Hungarian cuisine it is added to goulash, and in Norwegian cuisine and Swedish cuisine it is used for making caraway black bread.[7], In Hungary and Serbia, caraway is commonly sprinkled over home-made salty scones (*köményes pogácsa* / *pogačice s kimom*). It is also used to add flavor to cheeses such as *bondost*, *pultost*, *havarti*, and *Titlist*.

Caraway seeds are also frequently used as a spice in Switzerland and in Alsace, where it is commonly called *cumin*. This may cause confusion as the smell and taste of true cumin is very different. Caraway oil is used to for the production of Kümmel liquor in Germany and Russia, Scandinavian akvavit, Icelandic brennivín. [7]

In Middle Eastern cuisine, caraway pudding, called *meghli*, is a popular dessert during Ramadan. It is typically made and served in the Levant area in winter and on the occasion of having a new baby. Caraway is also added to flavor *harissa*, a North African chili pepper paste. In Aleppian Syrian cuisine, it is used to make the sweet scones named *keleacha*. Caraway

fruit oil is also used as a fragrance component in soaps, lotions, and perfumes. Caraway is also used as a breath freshener, and it has a long tradition of use in folk medicine.

Medicinal activity.

In indigenous Arabic medicines, the seeds are documented as stimulant, carminative, and attributed with cooling affect and therefore form an ingredient of most prescriptions for gonorrhea, chronic diarrhea and dyspepsia; externally, they are applied in the form of poultice to allay pain and irritation of worms in the abdomen. Seeds reduced to powder, mixed with honey, salt and butter are applied to scorpion bites. In Poland, caraway is recommended as a remedy to cure indigestion, flatulence, lack of appetite, and as a galactagogue. In Russia, it is also used to treat pneumonia. In Great Britain and USA, it is regarded a stomachic and carminative. In Malay Peninsula, caraway is one important medicinal herb, and in Indonesia, it is used in the treatment of inflamed eczema [8]

Experimental

1- Processing of *Carum carvi*

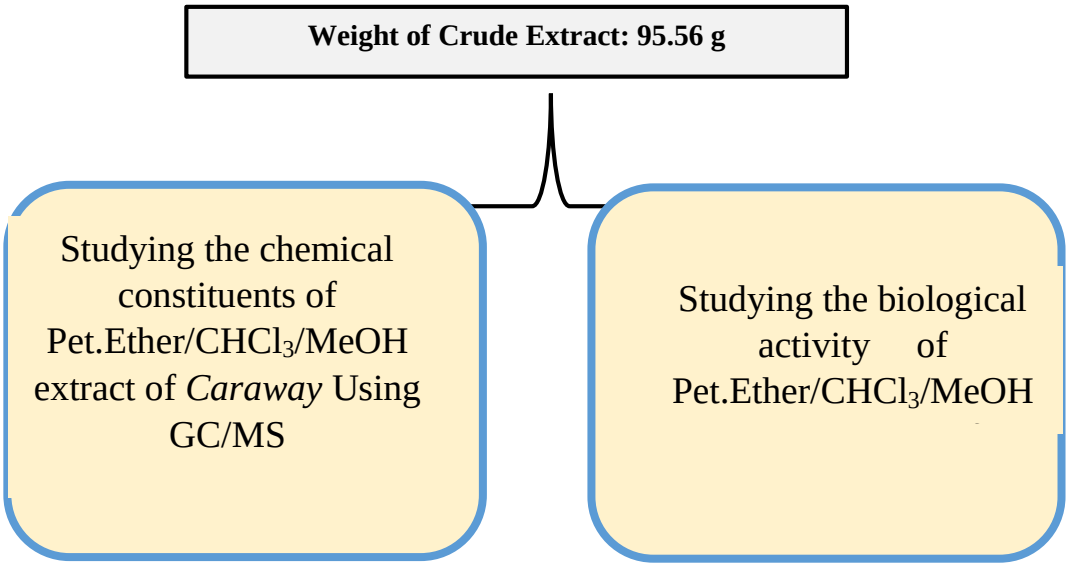
The parts of plant *Carum carvi* 200.0 g was dried in the shade at room temperature 22 ℃ and grinded well, then extracted with equal volumes of Pet.Ether/$CHCl_3$/MeOH (3 × 1 L) at room temperature for 2 weeks. Evaporation of the solvents under reduced pressure by rotary evaporator provided 95.56 g oily residue respectively

2-Applying Liquid-Liquid Extraction technique for the plant *Carum carvi.*

71.67 g of the crude Pet.Ether/$CHCl_3$/MeOH extract of Carum carvi dissolved well in aqueous solvent (H2O), then extracted well three times by separating funnel using three different organic solvents (Pet. Ether, Chloroform and Ethyl Acetate) to afford three fractions (**A**-**C**).

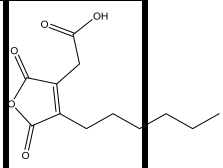
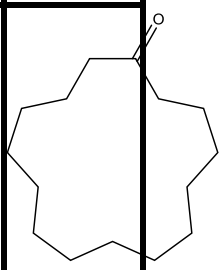
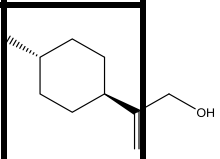
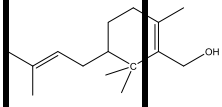
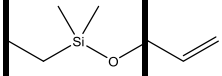
- Pet.Ether fraction (**A**): 20.0 g
- Chloroform fraction (**B**): 33.00 g
-Ethyl Acetate fraction (**C**): 18.67

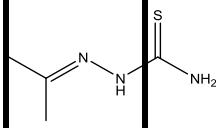
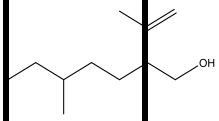
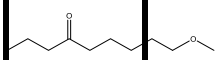
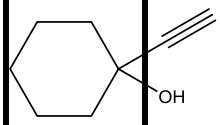
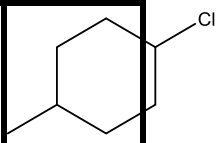
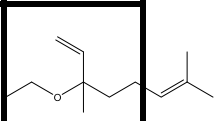
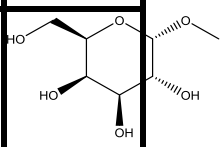
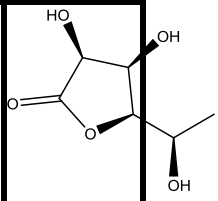
1. (200.0 g) dry plant.
2. Extraction by Pet.Ether. $CHCl_3$: MeOH (1:1:1) three times.
3. Filtration and Evaporation the solvent under reduced pressure to dryness.

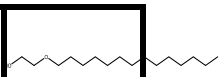
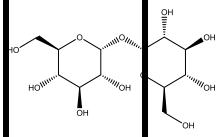
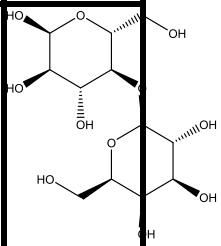
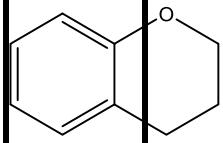
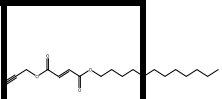
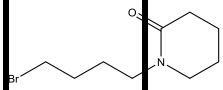


- In addition, the Pet.Ether/$CHCl_3$/MeOH extract of, *Carum carvi*, was identified by GC/MS technique, only **25** compounds were showed by their analogous reported by NIST.
- The biological activity of these fractions has been studied, and the major compounds of the crude Pet.Ether/ $CHCl_3$ /MeOH extract of Carum carvi have been identified using GC/MS.

Table1 Chemical constituents identified by GC/MS technique from Pet.Ether/$CHCl_3$/MeOH extract of *Caraway* (*Carum carvi*) whole plant material

No	Compound Name	Rt	Mol. wt	Area %	MS-Data	CSBC
1	3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo-	5.85	240	4.10	196(5),168(8),151(3)126µ+(100%), 98(22),81(11),65(9),44(54),27(15).	
2	Cyclopentadecanone	6.323	224	0.63	224(9),166(4),149(6),125(18),96(32),71(80),55µ+(100%),29(24).	
3	Cyclohexane ethanol, 4-methyl-. beta. -methylene-, trans-	6.918	154.3	0.55	136(16),121(19),107(18),96(44), 81(73),67(35),55µ+(100%),41(46), 29(20).	
4	1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene	7.12	222	0.36	207(7),189(7),161(6),135(22),121(32),107(41),93(56),81(53), 69µ+(100%).	
5	Ethyl(dimethyl) allyloxysilane	7.33	144	0.7	144(3),129(9),115µ+(100%),101(16) 85(23),75(16),59(50),45(16,27(5).	

6	Hydrazinecarbothioamide, 2-(1-methylethylidene)-	7.605	131	0.55	131µ+(100%),116(55),89(19),72(12), 57(57),43(43).	
7	3-Methyl-2-(3-methylpentyl)-3-buten-1-ol	7.811	170	1.45	170(3),152(10),137(17),123(10),109 (26),69(19),83(73),71µ+(100%,55(90)41(54),29(26).	
9	4-Nonanone, 9-methoxy-	8.074	172	0.77	172(3),140(11),129(17),112(11),97(46),86(34), 71µ+(100),58(58),45(93).	
11	*Cyclohexanol, 1-ethynyl-*	8.457	124	18.68	123(8),109(20),95(59),81µ+(100),68(68),53(27),39(40),27(28).	
12	1-Chloro-4-methylcyclohexane	8.64	132	6.15	96(30),81µ+(100),67(29),55(49),41(24), 27(10).	
13	1,6-Octadiene, 3-ethoxy-3,7-dimethyl-	8.75	182	10.69	150(4),137(4),121(26),107(5),96(42),83 (40),71(93),55(62),43µ+(100).	
14	.alpha.-D-Galactopyranoside, methyl	8.938	194	37.73	145(3),131(2),116(3),97(4),85(6),74(72), ,60µ+(100),43(36),33(13).	
15	6-Deoxy-D-mannono-4-lactone	10.958	162	1.43	144(4),118(9),100(29),87(12),72(67).60 (45),45µ+(100),29(38),15(21).	

16	Ethanol, 2-(tetradecyloxy)-	11.044	258	1.41	227(4),196(9),168(9),139(5),111(26), 83(60),57µ+(100),29(21).	
17	.alpha.-D-Glucopyranoside, .alpha.-D-glucopyranosyl	11.124	342	2.77	163(4),146(5),126(3),97(13),73µ+(100), 44(36),28(16).	
18	Lactose	11.965	342	0.15	145(2),126(4),97(7), 73µ+(100),57(76),29(20).	
19	2H-1-Benzopyran, 3,4-dihydro-	14.454	134	0.52	134µ+(100),119(14),106(16),91(16),78(30),63(2),51(9).	
20	Ethanol, 2-(octadecyloxy)-	15.284	314	0.08	283(3),252(3),224(4)141(4),111(16),85(45) 57µ+(100),29(26).	
21	Fumaric acid, dodecyl propargyl ester	15.467	322	0.09	266(4),237(3),209(12),179(6),155µ+(100), 125(14),99(78),69(76),43(40).	
22	2-Piperidinone, N-[4-bromo-n-butyl]-	15.57	233	0.02	112(3.25),97(13),69(24),43µ+(100).	
23	Tetracosane	26.727	388	4.70	388(8),281(3),253(1),225(2),197(3),169(3), 141(5),113(15),85(52),57 µ+(100).	

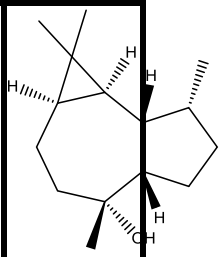
24	Octadecane	36.089	254	5.22	169(3),141(4),113(9),85(42),57µ+(100),29(18).	
25	Ledol	36,403	222	0.01	222(3),204(7),189(8),161(17),133(10),109(42), 93(33),69(58),43µ+(100),27(26).	

Table (2) Antibacterial activities of Carum carvi plant extract against tested bacterial organism (mean±SD)

Carum carvi	**1**	**0**	**2 ± 0.06**	**0**	**0**	**0**
Pet-Ether extract	**10**	**0**	**3 ± 0.12**	**0**	**0**	**0**
	25	**0**	**5 ± 0.17**	**0**	**0**	**0**
	50	**0**	**6 ± 0.29**	**0**	**0**	**0**
Carum carvi	**1**	**0**	**1 ± 0**	**1 ± 0**	**0**	**2 ± 0.12**
Chloroform extract	**10**	**0**	**3 ± 0.12**	**3 ± 0.12**	**0**	**3 ± 0.12**
	25	**0**	**4 ± 0.29**	**5 ± 0.29**	**0**	**5 ± 0.29**
	50	**0**	**5 ± 0.17**	**6 ± 0.29**	**0**	**6 ± 0.29**
Carum carvi	**1**	**0**	**0**	**0**	**0**	**0**
ETOAC extract	**10**	**0**	**0**	**0**	**0**	**0**
	25	**0**	**0**	**0**	**0**	**0**
	50	**1 ± 0.12**	**0**	**0**	**0**	**0**

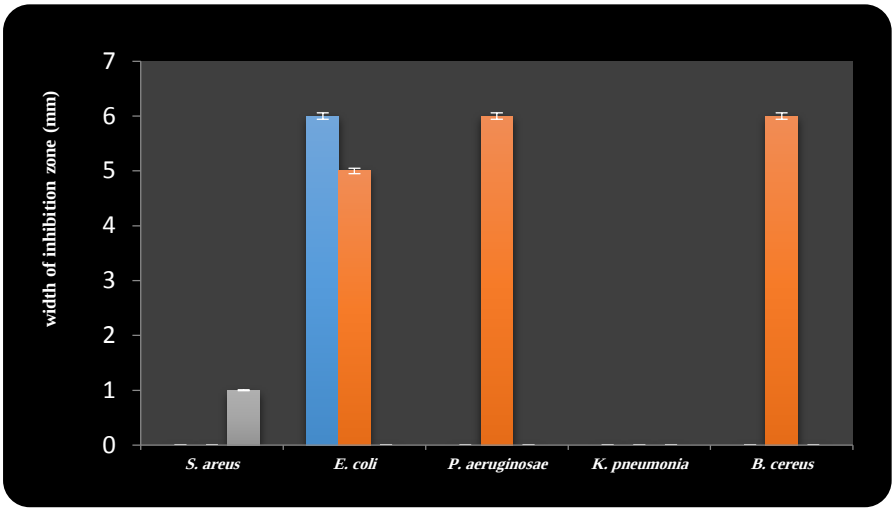


Figure (3). Antibacterial activities of different extracts at concentration 50 mg/ml from *Carum carvi.*

Caraway essential oil performed medium antimicrobial activity, although it inhibits growth of many bacteria. Table (2) indicated that, chloroform extract exhibited medium antibacterial activity against *E. coli*, *P. aeruginosae* and *B. cereus* (3 - 6 mm). Increasing the chloroform extract concentration and the greater effect on the *E. coli*, *P. aeruginosae* and *B. cereus* (50mg/ml) growth inhibition occurred (Figure 3). While petroleum ether extract of *Carum carvi* have antibacterial inhibitory effect only against *E. coli*, and ETOAC

According to [112] *Carum carvi* showed a non-negligible antimicrobial activity, probably related to the richness of corvone and limonene, these monoterpenes are known for their antiseptic activity. and all *Carum carvi* extracts (Pet-Ether, Chloroform and ETOAC) did not show any noticeable activity against all tested fungi in all concentrations.

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