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Evaluation of Antibacterial Activity, Gas Chromatography Analysis and Antioxidant Efficacy of Artichoke (*Cynara scolymus L.)*

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ABSTRACT

Artichoke a perennial thistle of the *Asteraceae* plant family, which is commonly eaten as a vegetable in North America, Europe and the Mediterranean. Ethanolic and methanolic extracts of artichoke leaves and pulp were prepared and tested for their antibacterial activity against eight bacterial strains: three Gram-negative bacteria (*E. coli, Pseudomonas aerugenosa and Salmonella* sp.) and five Gram-positive bacteria (*Bacillus cereus ,Bacillus subtilis , Listeria monocytogenes , Streptococcus faecalis* and *Staphylococcus aureus*). The leaves and pulp parts were also tested for their antioxidant capacity, total phenols and total flavonoids content. The phytochemical composition of artichoke leaves and pulp were qualitatively determined. The data of the antibacterial activity test revealed that the highest inhibitory effect was recorded for ethanolic leaves extract against Gram-positive bacteria *Staphylococcus aureus* (18 mm.) ,while the ethanolic pulps extract exerted the same inhibitory activity against the Gram positive bacteria *B. subtilis* and Gram negative bacteria *Pseudomonase aeroginosa* with inhibition zone diameters of 12.0 mm .Phytochemical analysis of artichoke pulp and leaves ensured that both parts are reservoirs of flavonoids and phenolic compounds that modulated its antioxidant activity. Qualitative determination of tannins, alkaloids and saponnins ascertained its existence in both leaves and pulp. Glycosids was found in leaves , and undetected in the pulp. The results showed that artichoke leaves had higher flavonoids, phenolics and antioxidant capacity than the pulp.

keywords: Artichoke, antibacterial activity, flavonoids, phenolic contents, antioxidant activity.

INTRODUCTION

Plants are bio-resource of nutraceuticals, food supplements and pharmaceutical intermediates for synthetic drugs (Ncube *et al*., 2008). Some plants such as artichoke (*Cynara scolymus L.*) leaves had long been used effectively for treating a variety of diseases. Artichoke contains caffeoylquinic acid derivatives (cynarin and chlorogenic acids) and flavonoids such as luteolin and apigenin (Llorach *et al.,* 2002).

Historically, this plant has been used in folk medicine since Roman time, for its health benefits which are mainly attributed to its high content of polyphenols and inulin (Pandino *et al.,* 2011). These substances are very important for the human nutrition since they are involved in the prevention of cancer (Clifford and Brown, 2006). Among the common edible plants, artichoke is the richest source of dietary antioxidants (Brown and Rice-Evans, 1998).

The present work describes the antibacterial activity of ethanolic and methanolic extracts against eight bacterial strains , as well as Gas chromatography analysis , phytochemical screening and antioxidant assays of artichoke leaves and pulps.

MATERIALS AND METHODS

Plant material

Artichoke plant was purchased from local market and plants were separated into leaves and pulps. The leaves were oven dried at 40 ºC overnight and fresh pulps were stored refrigerated in plastic polyethylene bags till further analysis.

Preparation of extracts

Ethanolic and methanolic extracts of both artichoke leaves and pulps were prepared by maceration method following the procedure described earlier by El-Chaghaby *et al*., 2014.

Antibacterial activity test

Methanolic and ethanolic extracts were screened for antibacterial activity against eight bacterial strains: three Gram-negative bacteria (*E. coli, Pseudomonas aeroginosa* and *salmonella* sp.) and five Gram-positive bacteria (*Bacillus cereus , Bacillus subtilis ,Listeria monocytogenes, Streptococcus faecalis* and *Staphylococcus aureus*). The tested bacteria were grown in buffered peptone water (pH 7.2) and incubated for 24 h at 37°C to achieve viable cell count of 10^8 cfu/ml.

The antibacterial activity of the extracts was carried out by disc diffusion method according to NCCLS (1993). Mueller-Hinton agar was the selected media for preparing the test plates. 100µl of the microbial suspension was taken and spread onto Mueller-Hinton agar. The disc papers were impregnated with 10 µl of tested extracts and placed on agar media seeded with the above mentioned bacteria.

Negative control was included using ethanol 90% as described by William. (1989).The inoculated plates were incubated at 37°C for 48 h. All tests were performed in triplicate.

Gas Chromatography-Mass Spectrometry Analysis:

The analysis was carried out using GC (Agilent Tecnologies 7890A) connected to a mass-selective detector (MSD, Agilent 7000). The flow of helium used as carrier gas was retained at 1 ml/min during the run (Patricia *et al.,* 2013). The components were confirmed by coordinating their mass spectra and retention time with the database of National Institute of Standard and Technology (NIST) library. The names, molecular weights and chemical structure of each of the components of the test materials were determined.

Phytochemical Screening tests

The extracts were subjected to phytochemical tests for plant secondary metabolites, tannins,

glycosides, alkaloids and saponins following the methods of Harborne (1973) and Evans (1996).

Determination of total antioxidant activity [TAA]

The total antioxidant activity of the extracts was determined using the phosphomolybdenum method according to the procedure described by (Prieto *et al.,* 1999). The antioxidant activity was calculated using a standard curve of ascorbic acid.

Determination of total phenolic Contents [TPC]

Contents of total phenolics of the extracts were estimated spectrophotometrically using the Folin–Ciocalteu assay (Singleton and Rossi, 1965). A standard curve was plotted using different concentrations of Gallic acid. The absorbance obtained was converted to gallic acid *equivalent* in mg per gm of dry material [mg GAE/g] using gallic acid standard curve.

Determination of total flavonoids (TF)

Total flavonoids content was determined as described by the method of Willet (2002). Total flavonoids content results were reported as equivalents to querectin used as standard.

Antibacterial activity

The data obtained in Table (1) cleared that ethanolic extract of artichoke pulp showed more antibacterial activity against the tested bacteria than the methanolic extract wherease the ethanolic extract exert antibacterial activity against gram positive bacteria (*B. subtilis ,Streptococcus faecalis* ,and *Staph aureus*) and gram negative bacteria (*Pseudomonas aerugenosa* and *E. coli*) expressed as inhibition zone diameters 12.0 mm, 12.0 mm ,10. 0 mm and 12.0 mm and 10.0 mm respectively on the other hand the pulp methanolic extract can inhibit only the growth of gram positive bacteria *Streptococccus faecalis* and gram negative bacteria *Pseudomonas aerugenosa* with inhibition zone 10.0 mm for each of them. Regarding to leaves ethanolic extract, it is obvious that the highest antibacterial activity (18.0 mm and 11.0 mm) was recorded against *Staph.aureus* (gram positive bacteria) and *Pseudomonas aerugenosa* (gram negative bacteria) respectively. On the contrary any of artichoke extracts (pulp and leaves) can't inhibit any growth of the gram negative microorganism *Salmonella* spp.

RESULTS AND DISCUSSION

Table 1. Antibacterial activity of artichoke pulp and leaves extracts.

Microorganisms / Sample	Gram positive bacteria					Grame negative bacteria		
	B. cereus	*B. subtilis*	*St. faecalis*	*L. monocytogenes*	*Staph. aureus*	*P.aerugenosa*	*E. coli*	*Salmonella* spp.
	Inhibition zone diameters (mm)							
Pulp Ethanolic extract	0.0	12.0	12.0	0.0	10.0	12.0	10.0	0.0
Pulp Methanolic extract	0.0	0.0	10.0	0.0	0.0	10.0	0.0	0.0
Leaves Ethanolic extract	7.0	10.0	11.0	10.0	18.0	11.0	10.0	0.0
Leaves Methanolic extract	7.0	9.0	9.0	10.0	9.0	10.0	9.0	0.0

○ **Average of triplicate determinations B:Bacillus St: Streptococcus L: Listeria Staph: Staphylococcus P: Pseudomonas E: Escherichia**

Gas Chromatography-Mass Spectrometry

The different biological activities (antibacterial and antioxidant activities) of prepared artichoke extracts are mainly attributes to their chemical composition. Thus mainly these extracts contain different organic compounds of different functional effective groups like phenolic groups , ,carboxylic groups, methoxy groups , aliphatic and secondary alcoholic groups , olefinic groups as well as many different other functional groups. The synergestic effect of these biologically effective groups plays an important role in different observed biological effect. Tables (2- 5) and Fig. (1-2) describe the different patterns of organic compounds that possess the previously mentioned functional groups as obtained from qualitative GC analysis. The peak area column in these tables illustrates the relative concentration as proportional to relative area.

Table 2. Gas Chromatography-Mass Spectrometry Analysis of artichoke pulp ethanolic extract.

No.	R.T.[a]	Name	peak area %
1	8.945	4-Hydroxy-7-methoxycoumarin	13.05
2	9.145	3,4,5-Trimethoxycinnamic acid	8.58
3	9.23	5,3'-Dihydroxy-6,7,4'-trimethoxyflavone	18.22
4	9.365	Luteolin	23.48
5	9.792	Isovitexin	2.05
6	10.05	Lanceol, cis	4.33
7	10.14	Nerolidol	1.91
8	11.24	Gentisic acid	3.05
9	11.54	Curcumin	2.25
10	12.23	Homoeriodictyol	0.58
11	12.55	p-Cresol, 2,2'-methylenebis[6-tert-butyl-	3.41
12	12.61	Salicylic acid	1.36
13	13.7	Tetra-O-methylfisetin	4.76
14	14.75	Coniferyl aldehyde	4.04
15	14.84	Scutellarein	1.21
16	15.59	Quercetin 3'-methyl ether	1.72
17	15.86	Baicalein trimethyl ether	2.69
18	16.16	Homobutein	0.98
19	17.28	Gardenin	1
20	20.36	Peonidin cation	1.33

R.T.[a]: Retention time of different peaks GC chromogram in minutes.

Table 3. Gas Chromatography-Mass Spectrometry Analysis of artichoke pulp methanolic extract .

No.	R.T.[a]	Name	Peak area %
1	3.213	Ethyl 8-methoxycoumarin-3-carboxylate	15.04
2	6.863	Avenanthramide-C methyl ester	40.11
3	8.973	4-Hydroxy-7-methoxycoumarin	19.91
4	9.022	Resodiacetophenone	1.88
5	9.14	Morin	2.84
6	9.185	3,5,7,3',4'-Pentahydroxyflavanone	0.96
7	9.1	2,5-Dihydroxyphenol	0.94
8	9.254	Cumic aldehyde	0.94
9	9.324	Luteolin	2.85
10	9.772	2'-Methoxy-6-methylflavone	1.44
11	10.05	Phloroglucinol	1.02
12	10.61	4-Methylesculetin	1.46
13	11.97	Syringic acid	1.85
14	13.19	Tetramethyl-O-scutellarin	0.63
15	14.29	Probucol	0.64
16	14.41	3-Hydroxy-7,8,2',3'-tetramethoxyflavone	1.29
17	14.7	4',6-Dimethoxyisoflavone-7-O-β-D-glucopyranoside	1.40
18	15.34	Vitexin	0.70
19	15.58	Methyl farnesate	0.87
20	15.8	7,8,4'-Trimethoxyisoflavone	1.23
21	15.99	Quercetin 3'-methyl ether	1.31
22	16.58	7-Hydroxy-4'-methoxyflavone	0.70

R.T.[a] : Retention time of different peaks GC chromogram.

Table 4. Gas Chromatography-Mass Spectrometry Analysis of artichoke leaves ethanolic extract .

No.	R.T.[a]	Name	Peak area %
1	9.145	3,4,5-Trimethoxycinnamic acid	20.02
2	9.381	Fraxidin	8.50
3	9.365	Luteolin	13.78
4	9.792	Isovitexin	2.47
5	10.05	Lanceol, cis	3.55
6	11.24	Gentisic acid	3.62
7	11.41	5,4'-Dihydroxyflavone	3.21
8	11.53	Datiscetin	3.24
9	11.54	Curcumin	1.93
10	11.78	Resorcinol	3.99
11	11.92	n-Propyl gallate	4.14
12	12.23	Homoeriodictyol	1.79
13	12.55	p-Cresol, 2,2'-methylenebis[6-tert-butyl-	2.92
14	12.61	Salicylic acid	11.25
15	13.7	Tetra-O-methylfisetin	2.23
16	14.75	Coniferyl aldehyde	5.19
17	14.84	Scutellarein	2.26
18	15.59	Quercetin 3'-methyl ether	1.53
19	15.86	Baicalein trimethyl ether	3.13
20	16.16	Homobutein	1.00
21	17.28	Gardenin	0.26

R.T.[a] : Retention time of different peaks GC chromogram.

Table 5. Gas Chromatography-Mass Spectrometry Analysis of artichoke leaves methanolic extract .

No.	R.T.[a]	Name	Peak area %
1	3.213	Ethyl 8-methoxycoumarin-3-carboxylate	32.94
2	6.863	Avenanthramide-C methyl ester	37.78
3	8.973	4-Hydroxy-7-methoxycoumarin	4.21
4	9.022	Resodiacetophenone	4.36
5	9.14	Morin	1.00
6	9.185	3,5,7,3',4'-Pentahydroxyflavanone	1.64
7	9.1	2,5-Dihydroxyphenol	1.15
8	9.254	Cumic aldehyde	0.71
9	9.324	Luteolin	1.78
10	9.772	2'-Methoxy-6-methylflavone	0.95
11	10.05	Phloroglucinol	0.59
12	10.61	4-Methylesculetin	0.93
13	11.97	Syringic acid	1.15
14	13.19	Tetramethyl-O-scutellarin	2.00
15	14.29	Probucol	0.58
16	14.41	3-Hydroxy-7,8,2',3'-tetramethoxyflavone	1.48
17	14.7	4',6-Dimethoxyisoflavone-7-O-β-D-glucopyranoside	1.83
18	15.34	Vitexin	0.43
19	15.58	Methyl farnesate	1.06
20	15.8	7,8,4'-Trimethoxyisoflavone	0.51
21	15.99	Quercetin 3'-methyl ether	1.90
22	17.17	Homobutein	1.01

R.T.[a] : Retention time in of different peaks GC chromogram

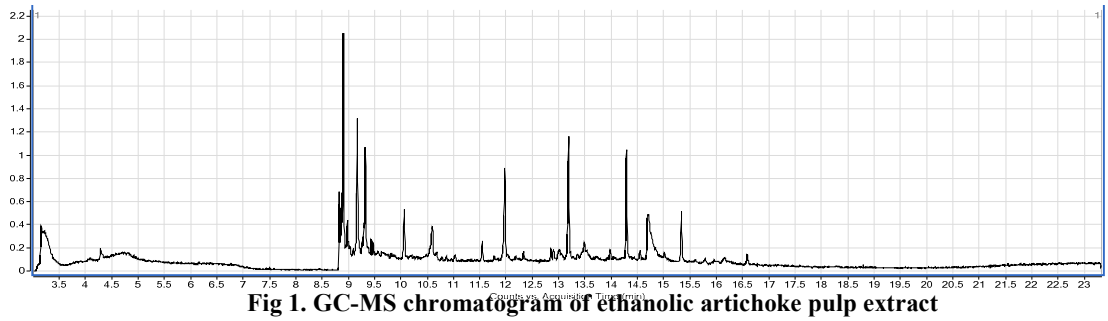

Fig 1. GC-MS chromatogram of ethanolic artichoke pulp extract

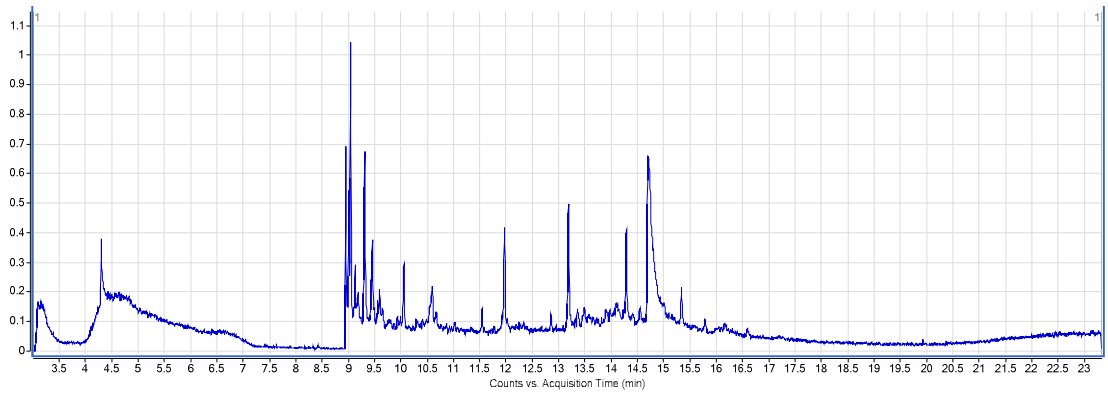

Fig 2. GC-MS chromatogram of ethanolic artichoke leaves extract

The GC/Ms analysis results showed the presence of some important organic compounds in the studied extracts. The main compounds identified include luteolin, Avenanthramide-C methyl ester, Ethyl 8-methoxycoumarin-3-carboxylate, Trimethoxycinnamic acid, 4-Hydroxy-7-methoxycoumarin and 5,3'-Dihydroxy-6,7,4'-trimethoxyflavone (Fig 3-7).

Luteolin is a flavone, a type of flavonoid. It is most often found in leaves, but it is also seen in rinds, barks, clover blossom, and ragweed pollen Mann (1992).

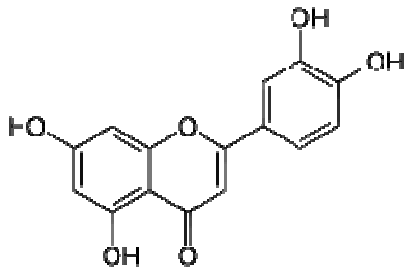

Fig 3.Leteolin

Qian Wang and Ming Jie (2010) declared that Luteolin showed obvious antibacterial activities against *Staph. aureus* , the antibacterial mechanism of luteolin is that it could inhibit the activity of DNA topisomerase I and II , which resulted in some decrease in the nucleic acid and protein synthesis .Xianfeng *et al.,* (2004) reported that luteolin -7- rutinoside and some other phenolic compounds from leaf extract of artichoke exhibited a relatively higher activity than other components against seven bacteria species .

Another main compound identified in the extracts is avenanthramide-C methyl ester, a polyphenol with proven anti-inflamatory and anti-cancer effects.

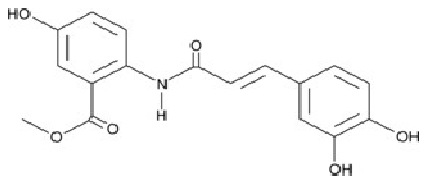

Fig 4. avenanthramide-C methyl ester

Alaa and Zeinab, (2013), obtained that maximum antibacterial activity was observed with methanolic extract of pound phenols for artichoke (bract and heart) against gram negative bacteria .YiFang Chu and Mitchell (2013) reported that avenanthramides are secondary metabolites that function as phytoalexins (antimicrobial compounds). They also possess potent antioxidant properties and have shown several interesting nutraceutical properties in laboratory tests. A number of studies demonstrate that these natural products have anti-inflammatory, antioxidant, anti-itch,anti-irritant, and antiatherogenic activities (Kurtz and Wallo 2007 & Koening *et al.,* 2011).

Also the extracts contain some coumarin derivatives (8-methoxycoumarin-3-carboxylate and 4-Hydroxy-7-methoxycoumarin)

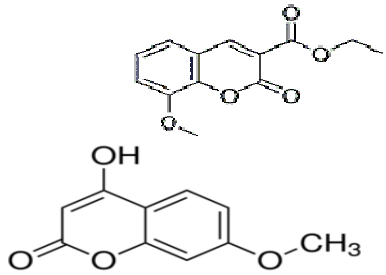

Fig 5. 8-methoxycoumarin-3-carboxylate and 4-Hydroxy-7-methoxycoumarin

Coumarin is naturally occurring plant constituent, it has been used in the treatment of cancer and oedemas, and many of its derivatives have also shown biological activity. Biological effects observed include antibacterial, anti-thrombotic and vasodilatory, anti-mutagenic and anti-tumourigenic effects as well as acting as lipoxygenase and cyclooxygenase inhibitors (Creaven *et al*., 2006). The substituents ester or carboxylic acid on the coumarin ring were needed to have potent inhibitory activity against both Gram-positive and Gram-negative bacteria (Kawase *et al*., 2011).

The compound trimethoxycinnamic acid was also identified in the extracts, it is a natural organic acid. Derivatives of cinnamic acid are common in plants. Methoxycinnamic acid and its derivatives have been shown to have interesting and important biological properties including analgesic, antibacterial, anti-inflamatory and anti-cancer effects (Jae-Chul *et al.,* 2010).

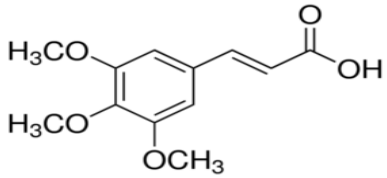

Fig 6. Trimethoxycinnamic acid

One of the main compounds that were also identified in artichoke extracts is: 5,3'-dihydroxy-6,7,4'-trimethoxyflavone. The compound is a flavonoid that was proven to have immense antimicrobial activity.

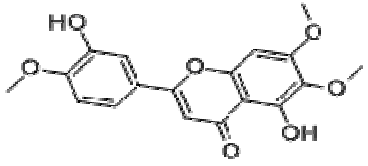

Fig 7. 5,3'-dihydroxy-6,7,4'-trimethoxyflavone

The results of GC/Ms analysis are in consistence with the results of antibacterial test. It has been proven that the studied extracts are composed of several compounds with effective antimicrobial effect.

Antioxidant activities

Antioxidant activities and qualitative determination of pytochemicals in artichoke leaves and pulp are shown in Tables (6and 7). It is clear that artichoke leaves had higher flavonoids, phenolics and antioxidant capacity than the pulp.

Table 6. Total antioxidant capacity (TAC), flavonoids (TF) and phenol content (TPC) of artichoke leaf and pulp

Plant part	TAC (ppm)	TF (ppm)	TPC (ppm)
Leaves	1580.62	644.25	791.0
Pulp	916.63	327.0	46.62

- **Results were expressed as average of three determinations**

Table 7. Qualitative detecation of tannins, glycosides, alkaloids and saponins

Test Plant part	Tannins	Glycosides	Alkaloids	Saponins
Leaves	++	+	++	++
Pulp	+	ND	+	+

ND: not detected

These data agree with Negro (2012) who reported that total phenolic content of artichoke is actually higher in leaves than in the pulps. Flavonoids in artichoke had antioxidative effects as reported by Jellin et al.(2002).

Menghini *et al.* (2010) had confirmed the ultimate free radical scavenging properties of artichoke leaf extract (ALE) and its major polyphenolic constituents.

Betancor-Fernandez *et al.* (2003) reported that aqueous extracts of artichoke leaf have the most interesting antioxidant properties, attributes to the presence of hydroxylcinnamates and flavonoid glucosides.

In the current study, analysis of artichoke pulps and leaves ensured that both parts are reservoirs of flavonoids and phenolic compounds that modulate their antioxidant activity. Phytochemical analysis ensured the presence of tannin, alkaloids and saponnins in leaf and pulp. Glycoside was detected in leaf, with negative result for the pulp.

Our results proved that both artichoke parts were rich in flavonoids, tannin, saponins and alkaloids, a fact that agree with Handa *et al.* (2008) who states that plants extracts contained alkaloids and flavonoids.

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تقييم النشاط المضاد للبكتيريا والتحليل الكروماتوجرافي الغازي والفعالية المضادة للأكسدة لنبات الخرشوف (سينارا سكوليموس)

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الخرشوف من النباتات المعمرة (عائلة أستيراسي) والتي عادة ما تؤكل كخضار في امريكا الشمالية و أوروبا ودول البحر المتوسط. تم اختبار المستخلصات الايثانولية والميثانولية لاوراق وقلب ثمرة نبات الخرشوف لمعرفة تأثيرها المضاد للبكتربا وهي عبارة عن ثلاثة ميكروبات سالبة لجرام (الايشيرشيا كولاي و السيدوموناس ايريجينوزا و السالمونيلا) وخمسة ميكروبات موجبة لجرام (باسيلس ساتيليس و باسيلس سيريس وستريبتوكوكس فيكاليس وليستيريا مونوسيتو جينس واستافيلوكوكس اوريس) . وتم أيضا تقدير المحتوي من مضادات الأكسدة والفينولات الكلية والفلافينويدات الكلية وكذلك تم تقييم المكونات الفيتوكيميائية في كل من مستخلصات الاوراق وقلب الثمرة . واشارت النتائج أن أعلي تأثير مضاد للبكتيريا تم تسجيله من خلال المستخلص الايثانولي للأوراق و كان ضد ميكروب أستافيلوكوكس أوريس (١٨ مم) بينما تساوي تأثير المستخلص الايثانولي لقلب الثمرة علي كل من ميكروب الباسيلس ساتيليس و ميكروب سيدوموناس ايريجينوزا (١٢ مم) . أثبتت نتائج التحليل الفيتوكيميائي أن كل من أوراق وقلب الثمرة عبارة عن مخزن للفلافونيدات والفينولات الكلية والتي تشكل نشاطا مضاد للأكسدة . أيضا تاكد وجود كلا من التانينات و القلويدات والصابونين في كل من الأوراق وقلب الثمرة .بالنسبة للجليكوسيدات تم تواجدها في مستخلصات الأوراق ولم تتواجد في مستخلصات قلب الثمرة . بينما أظهرت النتائج أن أوراق نبات الخرشوف كانت أعلي في محتوي الفلافونيدات والفينولات الكلية وكذلك مضادات الأكسدة من الثمرة