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Phytochemical, HPLC Analysis and Antibacterial Activity of Crude Methanolic and Aqueous Extracts for Some Medicinal Plant Flowers.

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Abstract

Methanolic and aqueous extracts of C. cardunculus, A. millefolium, C. officinalis, and M. chamomilla flowers were Phytochemical, Identification of polyphenols and flavonoids by HPLC, and also investigated for their antibacterial activity, Escherichia coli, Staphylococcus aureus and Bacillus subtillis. The phytochemical was observed that crude methanolic and aqueous extracts of investigated flowers the highest content from activity complex. HPLC analysis identified eighteen polyphenolic compounds as authentic samples namely: Gallic acid, pyrogallol, 4-amino benzoic, protocatechuic, cataehein, chlorogenic, catechol, e.picatechen, caffien, p.oh.benzoic, caffeic, vanillic, ferulic, ellagic, benzoic acid, salicylic acid, coumarin and cinnamic acid. While, flavonoid compounds its eleven compounds as authentic samples namely: narengin, rutin, hisperdin, romarinic, quereitrin, quereetrin, narenginin, kampferol, luteolin, hispertin, and 7-Hydoxyflavon. The methanolic extracts of C. officinalis and M. chamomilla flowers produced the highest growth inhibition (43.88 and 42.11%) for against B. subtillis at 6 mg/ml, While, the aqueous extracts of C. officinalis and M. chamomilla flowers produced the highest growth inhibition (29.99 and 29.22 %) for against Bacillus subtillis at 6 mg/ml. Moreover, the C. officinalis and C. cardunculus flowers extract produced the highest growth inhibition for methanolic and aqueous extracts of against Escherichia coli at 6 mg/ml.

Keywords*: phytochemical, Hplc analysis, plant extracts , antibacterial activity*

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Introduction

The phytochemical constituents of crude methanolic and aqueous extracts for *Cynara cardunculus, Achillea millefolium, Calendula officinalis* and *Matricaria chamomilla* flowers **Haghi *et al.,* (2014)**. The methanolic extract was higher in focus than aqueous extract, where it is more polarity. Identified some polyphenolic derivatives from the flowers of *C. cardunculus,* for instance, **Falé *et al.,* 2014**, analyzed by (HPLC) resulted in the presence of the following chemical compounds, Chlorogenic acid. **Rigane *et al.,* 2013**, who found the five compounds, were identified and quantified by LC/MS and HPLC in *C. officinalis* leaves and flowers of aqueous-methanolic extracts, were Rutin, quercetin-3-O-glucoside, scopoletin-7-O-glucoside, isorhamnetin-3-O-glucoside and gallic acid. Likewise, **Haghi *et al.,* 2014**, found that 30 phenolic compounds in *Matricaria chamomilla* and its extracts by UPLC-UV, e.g. chlorogenic

acid, caffeic acid, p-coumaric acid, salicylic acid, rutin, apigenin-7-glucoside, quercetin, luteolin, apigenin, kaempferol and isorhamnetin, were the most predominant components. Identified some Flavonoids derivatives from the flowers of *C. cardunculus*, for instance, **Benetis *et al.,* 2008**, detected the content of the identified flavonoids, in *A. millefolium* flowers, flavone-O-glycosides, namely: apigenin-7-O-glucoside, luteolin-7-O-glucoside, and their corres ponding free aglycones apigenin and luteolin. Moreover, **Rigane *et al.,* 2013**, who found the some compounds were identified and quantified by LC/MS and HPLC in leaves and flowers of aqueous-methanolic extracts, Rutin, quercetin-3-O-glucoside, scopoletin-7-O-glucoside, and isorhamnetin-3-O-glucoside. Likewise, **Al-Bahtiti *et al.,* 2012**, who isolated and identified of *Matricaria chamomilla* flowers, by HPLC technique as flavone and flavonols compound namely: kaempferol-o-glycosides, quercetin-o-glycosides, and myricetin-o-glycosides. The effect of extracts as antibacterial agents, **Alaa and Zeinab, *et al.,* 2013**, they found that antimicrobial activities of free and bound methanolic extracts of different parts of Artichoke for heart bound phenols extract on *E. Coli, St. aureus* and *B. Subtilis*. Moreover, **Anupam *et al.,* 2011**, This plant showed good antibacterial activity for instance, Roopashree *et al.,* 2008, who found antibacterial activity of aqueous extracts for Calendula officinalis, for *E. coli, B. subtillis* and *St.coccus*. Likewise, **Al-Bahtiti *et al.,* 2012**, found the chamomile extracts have activity against *Escherichia coli, Staphylococcus aureus and Bacillus subtillis.*

Materials and methods

Plant materials

Flowers sample from each species were air dried in the shade and ground into a fine powder. The powdered air dried flowers were divided into two extracts: First extract: Powdered air dried flowers (2 Kg) from each plant were extracted by soaking at room temperature for six times with methanol (30 L), then the methanolic extracts were concentrated to nearly dryness under reduced pressure using the rotary evaporator at 45°C to achieve the crude methanol extract which kept for further investigation **(El-Khateeb *et al.,* 2014)**. The yields of extracts were 17.9%, 16.5%, 19.8% and 18.3%, for *C. cardunculus, A. millefolium, C. officinalis,* and *M. chamomilla*, respectively. Second extract: Powdered air dried Flowers (2 Kg) of dried samples were extracted with distilled water by boiling at temperature from 80 to 100°C in reflux for 3 h to achieve an initial extract. All extracts were filtered after cooling to room temperature. Finally, the extracts were lyophilized and preserved at −20°C until further use **(Kim *et al.,* 2011)**. The yield of aqueous extracts were 7.7%, 6.2%, 9.4% and 8.6%, for *C. cardunculus, A. millefolium, C. officinalis,* and *M. chamomilla*, respectively,

Preliminary phytochemical tests of crude methanolic and aqueous extracts of investigated Flowers:

Preliminary phytochemical tests were carried out on the crude methanolic and aqueous extracts by boiling for 3 hours, extract to detect the presence of: terpenes, tannins, flavonoids, saponins, alkaloids, carbohydrate and/or glycosides, phenolic glycosides and resins.

Detection of terpenes: Terpenes were detected according to method adopted by **Harborne (1988).** A small amount of crude aqueous plant extract was dissolved in chloroform, then a few drops of concentrated sulfuric acid were added carefully on the wall of test tube to form two separated layers, the resulted yellow ring changed to orange then red indicating the presence of terpenes.

Detection of tannins: Tannins were detected by the method described by **Harborne (1988).** Few milliliters of distilled water were added to few milliliters of aqueous extract and filtrate, then ferric chloride solution (5%) was added to the filtrate. The presence of tannins yellowish green color was obtained.

Detection of flavonoids: Flavonoids were detected according to **Harborne (1988).** A small amount of crude plant extract was macerated in hydrochloric acid (1%) over night, then

sodium hydroxide solution (10%) was added to the filtrate, the appearance of yellow color indicates the presence of flavonoids.

Detection of saponins: Saponins were detected according to **Harborne (1988).** The aqueous crude plant extract was vigorously shaken developing a voluminous froth which persisted for almost one hour indicate the presence of saponins.

Detection of carbohydrate and/or glycosides: Carbohydrate and/or glycosides in crude plant extract were detected using Molish's reagent according to **Harborne (1988).** Some drops of α-naphthol in ethyl alcohol were added to 1ml of crude Boiling Water extract, then 1ml of concentrated sulfuric acid was added carefully without shaking, a purple ring was appeared indicating the presence of carbohydrate and/or glycosides in crude plant extract.

Detection of alkaloids: Alkaloids were detected according to **Harborne (1988)** by adding 2ml of diluted hydrochloric acid to 1ml of plant extract. Then five drops of ***Wagner's reagent** were added to 1ml of the previous solution and shaking after addition of each drop. After leaving for sometimes, the formed precipitate indicating the presence of alkaloids.

Detection of phenolic glycosides: Phenolic glycosides were detected according to **Harborne (1988)** by the following technique: some drops of concentrated sulfuric acid were added to 1ml of plant extract, a red color was produced which disappear when water was added.

Detection of resins: Resins were detected according to the methods described by **Harborne (1988).** The crude boiling water extract was boiled on water bath for 20 minutes and distilled water was added to extract, a white precipitate was formed in presence of resins.

Analysis of polyphenols by HPLC technique:

Phenolic compounds were determined by HPLC according to the method of **Goupy *et al.,* (1999)**, as follow : 5 g of sample were mixed with methanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtered through a 0.2 µm Millipore membtrane filter then 1-3 ml was collected in a vial for injection into HPLC Agilent 1200 series equipped with auto sampling injector, solvent degasser, ultraviolet (UV) detector set at 280 nm and quarter HP pump (series 1050). The column temperature was maintained at 35°C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. Phenolic acid standard from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculation of phenolic compounds concentration by the data analysis of HEWLLET packed software.

Analysis of flavonoids by HPLC technique:

Flavonoid compounds were determined by HPLC according to the method of **mattila *et al*., (2000)**, as follow: 5 g of sample were mixed with methanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtered through a 0.2 µm Millipore membtrane filter then 1-3 ml was collected in a vial for injection into HPLC Agilent 1200 series equipped with auto samplling injector, solvent degasser, ultraviolet (UV) detector set at 254 nm and quarter HP pump (series 1050). The column temperature was maintained at 35°C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. Flavonoid acid standard from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculation of phenolic compounds concentration by the data analysis of HEWLLET packed software.

Crude methanolic and aqueous extracts of investigated flowers as Antibacterial activity:

To study the effect of investigated Flower extracts, three cultures of bacteria namely*: Escherichia coli, Staphylococcus aureus* and *Bacillus subtillis* were used according to **Shan *et al*., (2007)**. Briefly, nutrient agar media and 30 ml were poured in the each Petri plate. Three wells of diameter 0.7 centimeter cut in each plate with the help of cork borer and then sealed with nutrient agar. In each prepared plate, extracts of the same concentration were poured in all wells using micro-pipette and three concentrations were made of each plant extract e.g. concentration of methanolic and aqueous extracts of *Cynara cardunculus*, *Achillea millefolium, Calendula officinalis* and *Matricaria chamomilla* were (1.5 - 3 - 6 µg/ml),

in separate nutrient agar plates. Then, plates were incubated at 37˚C for 24 hrs. The diameter of inhibition zones were calculated as percentages.

Statistical analysis

Statistical analyses of all experimental data were done using the statistical software package **(CoStat, 2005).** All comparisons were first subjected to one way analysis of variance (ANOVA) and significant differences between treatment means were determined using Duncan's multiple range test at $P<0.05$ as the level of the significance (**Duncan, 1955)**.

Results and discussion

Preliminary phytochemical tests of crude aqueous and methanolic extracts of investigated flowers:

Table (1) represented the phytochemical constituents of crude aqueous and methanolic extracts for *Cynara cardunculus, Achillea millefolium, Calendula officinalis* and *Matricaria chamomilla* flowers. It could be observed that the crude aqueous and methanolic extracts of investigated flowers were rich in terpenes except *C. cardunculus* flowers, tannins, flavonoids, alkaloids, carbohydrate or glycosides and phenolic glycosides within the acceptable limits. But, all extracts were poor in resins. The methanolic extract was higher in focus than aqueous extract, where it is more polarity according to **Haghi *et al.,* (2014)**. Our data about *Cynara cardunculus* flowers were in agreement with those reported by **Bezakova *et al.,* (2007)**, who found that *Cynara cardunculus* flowers were rich in flavonoids and glycosides.

Also, present data about *Achillea millefolium* flowers extract was agreed with those described by **Vitalini *et al.,* (2011)**, who isolated and chemically identified peptide and cyclopeptide were flavonoids, tannins, saponin, and glycosides from *A. millefolium*.

The data about *Calendula officinalis* flowers were in agreement with those reported by **muley *et al.,* (2009), Chakraborthy, (2010**) and **Khalid *et al.,* (2012)**, who established that *Calendula officinalis* flowers were rich in flavonoids, terpenes, glycosides, tannins, and saponin in methanol extract.

The results of aqueous and methanolic extracts of *Matricaria chamomilla* flowers were agreed with those suggested by **Haghi *et al.,* (2014)** and **Al-Bahtiti *et al.,* (2012)**, who found that the highest amount of phenolic and flavonoid tannins and apigenin were detected in the methanol extract.

Table 1. Preliminary phytochemical tests of crude Aqueous and methanolic extract of investigated Flowers.

Plants	Extracts	Terpenes	Tannins	Flavonoids	Saponin	Glycosides	Alkaloids	phenolic glycosides	Resins
C. cardunculus	**Aqueous**	–	+++	+++	++	++	+++	–	–
	Methanolic	–	++	++	+	+	++	–	–
A. millefolium	**Aqueous**	+	+++	+++	++	++	–	–	–
	Methanolic	+	++	++	+	+	–	–	–
C. officinalis	**Aqueous**	+	+++	++	+++	++	+	+	–
	Methanolic	+	++	++	++	++	+	+	–
M. chamomilla	**Aqueous**	+	+++	+++	++	+++	–	++	–
	Methanolic	+	++	++	++	++	–	++	–

Analysis of polyphenols by HPLC:

High performance liquid chromatography (HPLC) procedure was used for qualitative and quantitative analysis of polyphenolic compounds in *Cynara cardunculus, Achillea*

millefolium, Calendula Officinalis and *Matricaria chamomilla* flowers. Eighteen polyphenolic compounds as authentic samples namely: Gallic, Pyrogallol, 4-Amino benzoic, Protocatechuic, Cataehein, Chlorogenic, Catechol, E.picatechen, Caffien, P.oh.benzoic, Caffeic, Vanillic, Ferulic, Ellagic, Benzoic acid, Salicylic acid, Coumarin and Cinnamic acid. These standard samples were used to identify the corresponding components in investigated flower polyphenols.

Obtained data revealed that seventeen compounds with different retention times were recognized in HPLC chromatogram of *Achillea millefolium*. While, sixteen compounds with different retention times were recognized in HPLC chromatogram of *Cynara cardunculus, Calendula officinalis* and *Matricaria chamomilla* flowers, respectively. From table (2), it is clear that all investigated flower samples contained 4-Aminobenzoic, Protocatechuic, Cataehein, Chlorogenic, Catechol, Caffien, Caffeic, Vanillic, Ferulic, Benzoic, Salicylic, Coumarin and Cinnamic acid with different concentrations comparing with standard compounds.

From table (2), it could be noticed that Gallic acid was the predominant identified component in *C. cardunculus*, *A. millefolium* and *C. officinalis* flowers in concentrations which were (70.06, 26.11 and 53.46 ppm), respectively. While, not found in *M. chamomilla* flowers but content pyrogallol (333.275 ppm) except *C. cardunculus*, *A. millefolium* and *C. officinalis*. The 4-Aminobenzoic content in *C. cardunculus*, *A. millefolium*, *C. officinalis* and *M. chamomilla* flowers which were (9.01, 15.54, 10.98 and 2.57 ppm), respectively. While, protocatechuic content in the same line which were (56.08, 79.27, 83.28 and 25.48 ppm), respectively. Also, the cataehein content in plants which were (15.68, 37.54, 11.73 and 10.69 ppm), respectively.

From table (2), it could be noticed that Chlorogenic was the predominant identified component in both *C. cardunculus* and *A. millefolium* flowers in concentrations of (96.32 and 170.35 ppm), followed by Catechol (23.96 and 35.54ppm). While, Catechol was the main component of polyphenols in *C. officinalis* flowers in concentration of (16.47 ppm) followed by Chlorogenic (42.91 ppm), While, Catechol in *M. chamomilla* flowers in concentration of (32.3969 ppm) followed by Chlorogenic (33.4703 ppm), E.picatechen (24.0297 ppm) While, was not found in *C. officinalis,* while They are present in *Cynara* and *Achillea* flowers, (36.22 and 83.9 ppm), respectively.

From table (2), it could be noticed that Ellagic was the predominant identified component in both *C. cardunculus* and *A. millefolium* flowers in concentrations of (94.42and 159.69ppm), followed by Benzoic (157.79 and 72.31 ppm). While, Benzoic was the main component of polyphenols in *C. officinalis* flowers in concentration of (483.98 ppm), followed by Ellagic (33.83ppm), While, Benzoic in *M. chamomilla* flowers in concentration of (340.516ppm) while, Ellagic not found in *Matricaria* flowers. The Salicylic content (130.848, 112.58, 68.79 and 24.0297ppm), in *C. cardunculus, A. millefolium, C. officinalis,* and *M. chamomilla* flowers, Respectively.

Data in table (2) showed that Pyrogallol and P.oh.benzoic was not found in *C. cardunculus.* While, most of the compounds containing other polyphenols. Pyrogallol and E.picatechen was not found in *C. officinalis,* Gallic and E.picatechen was not found in *M. chamomilla* flowers. The 4-Aminobenzoic, Protocatechuic, Cataehein, Chlorogenic, Catechol, Caffien, Caffeic, Vanillic, Ferulic, Benzoic, Salicylic, Coumarin and Cinnamic acid, all these compounds found high rates in all plants.

Several authors identified some polyphenolic derivatives from the flowers of *Cynara cardunculus,* for instance, **Christaki *et al.,* (2012)**, found to be rich in polyphenolic compounds, The main phenolic compounds were 5-O-caffeoylquinic acid (Chlorogenic acid) which was the most abundant single substance (39%), followed by 1,5-O-dicaffeoylquinic acid (21%) and 3,4-O-dicaffeoylquinic acid (11%), based on total caffeoylquinic acid content. Vitalini *et al.,* (2011), isolated pure Phenolic compounds 1,3-O-dicaffeoylquinic acid, Chlorogenic acid, Rutin and Luteoin-7-o-glucoside. As mentioned, by **Eghdami and sadeghi (2010)**, phenolic contents in *Achillea millefolium* methanolic extract was 123.9 mg GAE/gm. While, for aqueous extract was 41.2 mg GAE/gm.

Preethi *et al.,* (2006), found that *Calendula officinalis* flowers are rich in carotenoids of which flavoxanthin has been reported to be present at 28.5% of total carotenoids followed by luteoxanthin it also contain lycopene, b-carotene and coumarins. Also, **Rigane *et al.,* (2013)**, stated that five compounds, were identified and quantified by LC/MS and HPLC in leaves and flowers of aqueous-methanolic extracts of *C. officinalis* which were Rutin, quercetin-3-O-glucoside, scopoletin-7-O-glucoside, isorhamnetin-3-O-glucoside and gallic acid.

Haghi *et al.,* (2014), found that 30 phenolic compounds in *Matricaria chamomilla* and its extracts by UPLC-UV, e.g. chlorogenic acid, caffeic acid, p-coumaric acid, salicylic acid, rutin, apigenin-7-glucoside, quercetin, luteolin, apigenin, kaempferol, and isorhamnetin, were the most predominant components. The data in table (2) revealed that all plants *C. cardunculus, A. millefolium, C. officinalis,* and *M. chamomilla* flowers were rich in polyphenolic compounds contents.

Table 2. Identification of polyphenols of investigated flowers using HPLC technique.

Polyphenol Compounds	*C. cardunculus*		*A. millefolium*		*C. officinalis*		*M. chamomilla*	
	R_t	ppm	R_t	ppm	R_t	ppm	R_t	ppm
Gallic	7.023	70.06	7.077	26.11	7.050	53.46	--	--
Pyrogallol	--	--	--	--	--	--	7.070	333.275
4-Aminobenzoic	8.097	9.01	8.090	15.54	8.126	10.98	8.200	2.57051
Protocatechuic	8.448	56.08	8.393	79.27	8.437	83.28	8.420	25.4822
Cataehein	8.580	15.68	8.602	37.54	8.573	11.73	8.605	10.6926
Chlorogenic	9.157	96.32	9.157	170.35	9.180	42.91	9.129	33.4703
Catechol	9.405	23.96	9.395	35.54	9.413	16.47	9.504	32.3969
E.picatechen	9.650	36.22	9.739	83.94	--	--	9.693	24.0297
Caffien	9.797	22.74	9.841	18.59	9.805	14.51	9.766	10.5155
P.oh.benzoic	--	--	9.926	39.02	9.933	16.52	9.902	56.6691
Caffeic	10.261	28.73	10.281	20.61	10.337	15.13	10.307	12.7119
Vanillic	10.406	8.46	10.441	62.73	10.414	31.83	10.428	24.2998
Ferulic	12.080	8.46	12.048	19.03	12.083	3.34	12.064	4.53954
Ellagic	13.263	94.42	13.236	159.69	13.263	33.83	--	--
Benzoic	13.836	157.79	13.763	72.31	13.750	483.98	13.825	340.516
Salicylic	14.250	130.848	14.231	112.58	14.338	68.79	14.209	65.1621
Coumarin	14.437	5.11	14.415	8.43	14.433	4.81	14.410	24.5978
Cinnamic	15.702	4.91	15.740	3.22	15.862	22.33	15.770	10.0495

Analysis of flavonoids by used HPLC:

High performance liquid chromatography (HPLC) procedure was used for qualitative and quantitative analysis of flavonoids compounds in *Cynara cardunculus, Achillea millefolium, Calendula officinalis,* and *Matricaria chamomilla* flowers. Eleven Flavonoids fraction as authentic samples namely: Narengin, Rutin, Hisperdin, Romarinic, Quereitrin, Quereetrin, Narenginin, Kampferol, Luteolin, Hispertin, and 7-Hydoxyflavon were used different concentrations comparing with standard compounds.

Obtained data revealed that Ten compounds with different retention times were recognized in HPLC chromatogram of *A. millefolium,* and *C. officinalis* flowers, and Eleven compounds with different retention times were recognized in HPLC chromatogram of *C. cardunculus* and *M. chamomilla* flowers respectively. From table (3), it is clear that all investigated Flowers samples contained, Rutin, Hisperdin, Romarinic, Quereitrin, Quereetrin, Narenginin, Kampferol, Hispertin, and 7-Hydoxyflavon with different concentrations comparing with standard compounds.

From table (3), it could be noticed that Narengin was the predominant identified component in both *C. cardunculus* and *A. millefolium* flowers in concentrations of (511.74 and 335.93 ppm), followed by Rutin (140.34 and 107.37 ppm). While, Rutin was the main

component of Flavonoids in C. officinalis flowers in concentration of (101.36 ppm), except Narengin. While, Rutinin *M. chamomilla* flowers in concentration of (37.15 ppm), followed by Narengin (398.80 ppm). While, the highest value for Hisperdinin *M. chamomilla*, (7616.12 ppm), Followed by A. millefolium, (2331.10 ppm), Followed by *C. officinalis* and *C. cardunculus* flowers, (844.27 and 160.40 ppm), respectively.

From table (3), it could be noticed that highest value for Romarinic, in *M. chamomilla,* (554.14 ppm), While the low value in *Cynara,* (6.73 ppm), and *Achillea,* (8.63 ppm), And Calendula, (14.86 ppm), The content, Quereitrin and Quereetrin, (647.85 and 63.64 ppm), (152.08 and 35.37 ppm), (644.73 and 83.36 ppm) and (102.63 and 58.28 ppm), in *C. cardunculus, A. millefolium, C. officinalis* and *M. chamomilla* flowers, respectively. Where the values Quereitrin, higher compared to Quereetrin.

From table (3), it could be noticed that Narenginin was the predominant identified component in both *C. cardunculus and A. millefolium* flowers in concentrations of (12.23 and 18.73 ppm), followed by Kampferol, (45.02 and 175.9 4ppm). While, Kampferol was the main component of Flavonoids in *C. officinalis* flowers in concentration of (35.70 ppm), followed by Narenginin, (15.70 ppm), While, Kampferol in *M. chamomilla* flowers in concentration of (114.11 ppm) followed by Narenginin, (16.60 ppm). The Luteolin content (84.66, 20.23 and 11.62 ppm), in *C. cardunculus, C. officinalis* and *M. chamomilla* flowers, respectively. While, not found luteolin, in *A. millefolium* flowers.

From table (3), it could be noticed that The highest value for Hispertin, in C. officinalis, (229.06 ppm), While the lowest value in *M. chamomilla,* (8.53 ppm). While the highest value for 7-Hydoxyflavon, in *A. millefolium,* (178.43 ppm), While the lowest value in *M. chamomilla,* (3.56 ppm).

Data in table (3), showed that Narengin was not found in *C. officinalis* and Luteolin, was not found in *A. millefolium,* While most of the compounds containing flavonoids others. Rutin, Hisperdin, Romarinic, Quereitrin, Quereetrin, Narenginin, Kampferol, Hispertin, and 7-Hydoxyflavon, Each of these compounds there in all plants variably.

Several authors identified some Flavonoids derivatives from the flowers of *Cynara cardunculus,* for instance, **Bezakova *et al.,* (2007)**, found there are six flavonoids (apigenin, luteolin, apigenine-7-glucosid, luteoline-7-glucosid, apigenine-7-rutinosid, luteoline-7-rutinosid)and cynarin were isolated from *Cynara carunculus.* While, **Dalen, *et al.,* (2012)**, stated that, ethanolic extract of *Cynara contains,* cynarin, flavonoids, cynarosides, alsinarosides and luteolin.

Benetis *et al.,* (2008), identified flavonoids, in *Achillea millefolium* flowers, and stated that flavone-O-glycosides, namely: apigenin-7-O-glucoside, luteolin-7-O-glucoside, and their corresponding free aglycones apigenin and luteolin were identified. As mentioned, **Haroon *et al.,* (2014)**, studied antioxidant and cytotoxic activities of *Achillea millefolium,* they found that antioxidant activity for Quercetin and Di-hydroquercetin showed the best results with IC50 to be 2.5, 2.2 And 2.26 µg/ml, respectively.

Paşca *et al.,* (2013), who found that chemical composition of the *C. officinalis,* contain carotenoids, flavonoids and mucilage, in percentages of 7.5, 0.55 and 3.83%, respectively. Also, **Rigane *et al.,* (2013)**, showed that some compounds were identified and quantified by LC/MS and HPLC in leaves and flowers of aqueous-methanolic extracts namly, Rutin, quercetin-3-O-glucoside, scopoletin-7-O-glucoside, and isorhamnetin-3-O-glucoside,were dominant. **Al-Bahtiti *et al.,* (2012)**, isolated and by HPLC technique as flavone and flavonols compounds namely: kaempferol-o-glycosides, quercetin-o-glycosides, and myricetin-o-glycosides in concentrations of 10.1, 23.7 and 7.9 mg/g, respectively of *M. chamomilla* flowers.

Data in table (3), showed that all plants C. cardunculus, A. millefolium, C. officinalis, and M. chamomilla flowers, possess rich in content from flavonoids compounds, and reveled that were identified more from compounds such as, Narengin, Narenginin and Hispertin.

Table 3. Identification of Flavonoid fractions of investigated flowers using HPLC technique.

Flavonoid Compound	*C. cardunculus*		*A. millefolium*		*C. officinalis*		*M. chamomilla*	
	R_t	ppm	R_t	ppm	R_t	ppm	R_t	ppm
Narengin	12.438	511.74	12.513	335.93	--	--	12.528	398.80
Rutin	12.556	140.34	12.564	107.37	12.567	101.36	12.588	37.15
Hisperdin	12.816	160.40	12.677	2331.10	12.701	844.27	12.774	7616.12
Romarinic	12.932	6.73	12.908	8.63	12.997	14.86	12.981	554.14
Quereitrin	13.627	647.85	13.571	152.08	13.624	644.73	13.582	102.63
Quereetrin	15.180	63.64	15.201	35.37	15.210	83.36	15.165	58.28
Narenginin	15.439	12.23	15.541	18.73	15.450	15.70	15.520	16.60
Kampferol	15.667	45.02	15.753	175.94	15.690	35.70	15.661	114.11
Luteolin	15.776	84.66	--	--	15.747	20.23	15.748	11.62
Hispertin	15.860	42.93	15.880	22.56	15.863	229.06	15.827	8.53
7-Hydoxyflavon	17.883	6.90	17.862	178.43	17.889	10.10	17.767	3.56

Antibacterial activity for crude methanolic extract of investigated flowers:

The effect of various concentrations (1.5, 3 and 6 mg/ml) of Cynara *cardunculus, Achillea millefolium, Calendula officinalis* and *Matricaria chamomilla,* flowers crude methanolic and aqueous extracts on the growth inhibition of *Escherichia coli, Staphylococcus aureus* and *Bacillus subtillis,* are shown in table (4). It is clear that growth inhibition percentage increased gradually with increasing the concentration of the extracts for all microbial strains under investigation. The effect of various concentrations (1.5, 3 and 6 mg/ml) of *Cynara cardunculus, Achille amillefolium, Calendula officinalis* and *Matricaria chamomilla*, flowers crude methanolic and aqueous extracts on the growth inhibition of Escherichia coli, Staphylococcus aureus and Bacillus subtillis, are shown in table (4). It is clear that growth inhibition percentage increased gradually with increasing the concentration of the extracts for all microbial strains under investigation.

From table (4), it could be observed that *C. officinalis* and *M. chamomilla* flowers for methanolic extract produced the highest growth inhibition (43.88 and 42.11%) against *Bacillus subtillis* at 6 mg/ml. While, the percentages of growth inhibition for *C. cardunculus* and *A. millefolium* flowers extracts which were (26.09 and 19.15 %) against the same microbial strains and with the same concentration, respectively.

Moreover, the *C. officinalis* and *C. cardunculus* flowers extract produced the highest growth inhibition (33.16 and 31.12 %) against *Escherichia coli* at 6 mg/ml. While, the percentages of growth inhibition for *A. millefolium* and *M. chamomilla* flowers extract which were, (25.15 and 25.11 %) against the same microbial strains and with the same concentration, respectively.

From table (4), it could be observed that *C. officinalis* and *M. chamomilla* flowers for aqueous extract produced the highest growth inhibition (29.99 and 29.22 %) against *Bacillus subtillis* at 6 mg/ml. While, the percentages of growth inhibition of *C. cardunculus* and *A. millefolium* flowers extracts which were (23.46 and 16.28 %) against the same microbial strains and with the same concentration, respectively.

Moreover, the observed of *C. cardunculus*, *C. officinalis* and *M. chamomilla* flowers extract produced the highest growth inhibition (25.11, 25.36 and 25.15 %) against *St.coccus aureus* at 6 mg/ml. While, The lower growth inhibition of *A. millefolium* flowers extract was (15.86 %) for against the same microbial strains and with the same concentration, respectively.

From data in table (4) it could be concluded that the methanolic and aqueous extract of *Calendula* flowers was the most effective as antimicrobial agenst investigated strains. Furthermore, *Matricaria* flowers extract had got the second level as antimicrobial activity and followed by *Cynara* flowers. Whereas, the methanolic extract of *Achillea millefolium* flowers have less effectual for all antimicrobial inhibitors. This may be attributed to the presence of bioactive components having antimicrobial effect.

Results of several authors were agreed with that obtained, for instance, Alaa and **Zeinab, *et al.,* (2013)**, found that antimicrobial activities of free and bound methanolic extracts of different parts of *Artichoke* for heart bound phenols extract on *E. Coli*, *St. aureus* and *B. Subtilis,* were 24.4, 21.75, and 20.3%, respectively. While, for bract bound phenols extract on *E. coli*, *St. aureus* and *B. subtilis,* were 27.55, 24. 5 and 25.35%, respectively.

Alireza (2012), revealed that paralleled antimicrobial activity of methanol extract of two *Achillea* species (*A. santolina* and *A. pachycephala*) on gram positive bacteria, they found that inhibition effect of *E. coli* were 10 and 11%. While, of *B. subtillis* were 7 and 10%, Also, of *St. coccus,* were 5 and 6% respectively.

The results were highest compared with that obtained by, **Roopashree *et al.,* (2008)**, who found antibacterial activity of aqueous extracts for *Calendula officinalis*, for *E. coli, B. subtillis* and *St.coccus* were 11.56, 13.66 and 14.54%, consecutively.

Al-Bahtiti *et al.,* (2012), found the extracts have activity against *St. aurous, C. albicans, E. coli, B. pubescens* and *P. sylvestris.* The activity has been observed to be due to the tannins and a pigenin present in the extract. The results were also compared to comparable results obtained, by **Munir *et al.,* (2014)**, observed that ethanolic extract of *M. chamomilla* against *E. coli* using concentrations of 2.5, 5, 10 and 20 µg/ml were 20, 23.55, 25.77 and 31.22 %, respectively. While, for *St. aureus* were 33.2, 39.37, 44.68 and 48.36 %, respectively.

Table 4. Effect of crude methanolic and aqueous extracts of investigated Flowers of growth inhibition for *Escherichia coli, Staphylococcus aureus, Bacillus subtillis*.

Flowers extract	Extract	Concentration (mg/ml)	% of growth inhibition for :		
			Escherichia coli	*St.coccus aureus*	*Bacillus subtillis*
Cynara Cardunculus	**Methanolic**	**1.5**	**24.11**	**21.67**	**21.22**
		3	**26.45**	**23.99**	**23.65**
		6	**31.12**	**27.07**	**26.09**
	Aqueous	**1.5**	**22.71**	**19.3**	**18.89**
		3	**23.88**	**21.58**	**19.76**
		6	**25.45**	**25.11**	**23.46**
Achillea Millefolium	**Methanolic**	**1.5**	**19.13**	**12.23**	**11.75**
		3	**23.01**	**16.09**	**17.22**
		6	**25.15**	**17.06**	**19.15**
	Aqueous	**1.5**	**18.44**	**11.65**	**11.22**
		3	**21.09**	**14.31**	**15.63**
		6	**23.18**	**15.86**	**16.28**
Calendula officinalis	**Methanolic**	**1.5**	**25.66**	**23.17**	**35.95**
		3	**29.78**	**26.74**	**39.22**
		6	**33.16**	**29.55**	**43.88**
	Aqueous	**1.5**	**23.46**	**21.25**	**25.8**
		3	**25.41**	**23.52**	**27.18**
		6	**27.22**	**25.36**	**29.99**
Matricaria Chamomilla	**Methanolic**	**1.5**	**18.11**	**26.87**	**35.56**
		3	**22.88**	**27.25**	**39.11**
		6	**25.11**	**29.37**	**42.04**
	Aqueous	**1.5**	**17.9**	**22.31**	**24.11**
		3	**18.1**	**23.55**	**27.8**
		6	**20.23**	**25.15**	**29.22**

Conclusion

Phytochemical tests of investigated plants showed the highest content of Terpenes, Tannins, Flavonoids, Saponin, Glycosides, Alkaloids and phenolic glycosides. Medicinal plants containing high percentage of active compounds such as polyphenols and flavonoids. Methanolic extracts were the most effective bacterial inhibitor followed by aqueous extract for *C. officinalis, M. chamomilla, C. cardunculus* and *A. millefolium* flowers respectively.

References

Alaa, A. G. and Zeinab, A. S. (2013). Phenolic compounds from artichoke (*Cynara scolymus* L.) byproducts and their antimicrobial activities. J. of Bio, Agr. 3, 2224-3208.

Al-Bahtiti, N. H. (2012). Chemical analysis and biological activity of Jordanian *Chamomile* Extracts. Adv. J. Sci. Technol, 4, 22-25.

Alireza, M. D. (2012). Antimicrobial activity and chemical composition of essential oils of chamomile from Neyshabur, Iran. J. Med. Plants Res, 5, 820-824.

Benetis, R.; Radusiene, J. and Janulis, V. (2008). Variability of phenolic compounds in flowers of *Achillea millefolium* wild populations in Lithuania. Medicina (Kaunas) 10, 775-781.

Bezakova, L.; Grancai, D.; Oblozinsky, M.; Vanko, M.; Holkova, I.; Palikova, I. G. and Gaplovsky, M. (2007). effect of flavonoids and cynarine from *cynara carunculus* on lipoxygenase activity. Acta, J. Facult, Pharma, Univ, Comenian, 54, 48-53.

Chakraborthy, G. S. (2010). phytochemical screening of *Calendula officinalis* linn leaf extract by TLC. In. J. of Research in Ayurveda & Pharmacy, 1, 131-134.

Christaki, E.; Bonos, E. and Paneri, P. F. (2012). Nutritional and functional properties of cynara crops (Globe artichoke and Cardoon) and their potential applications international J. of Applied Science and Technology, 64-70.

CoStat program, Version 6.311 (2005). CoHort Software, 798 Lighthouse Ave. PMB 320, Monterey, CA, 3940, USA. http://www.cohort.com.

Dalen, H. M.; Nasser, A. and Mohamed, E. H. Agha. (2012). Study of the effect of leaves-ethanolic extract of Cynara, growing in Syria, on the total cholesterol and triglyceride levels in rats. Arab. J. Ph. Sci, 125-132.

Duncan, D. (1955). Multiply range and multiple F test. Biometrics, 11, 1-42.

Eghdami, A. and Sadeghi, F. (2010). Determination of total phenolic and flavonoids contents in methanolic and aqueous extract of *Achillea millefolium*. Org. Chem. J. 2, 81-84.

Falé, C. F.; Ana, M-R.; Fátima, N. F. and Maria, L. M. S. (2014). Studies on the molecular mechanism of cholesterol reduction by Fraxinus angustifolia, Peumus boldus, Cynara cardunculus and Pterospartum tridentatum infusions. J. Med. Plants Res, 9-17.

Goupy, P.; Hugues, M.; Biovin, P. and Amiot, M. J. (1999). Antioxidant composition and activity of barley (Hordeum vulgare) and malt extracts and isolated phenolic compounds. J. Sci. Food Agric, 1625-1634.

Haghi, G.; Hatami, A.; Safaei, A. and Mehran. M. (2014). Analysis of phenolic compounds in *Matricaria chamomilla* and its extracts by UPLC-UV. Res. in Pharma. Scin, 31-37.

Harborne, J. B. (1988). Phytochemical methods, 2nd ed. Published in USA by Chapman and Hall 29, west 35th street, New York.

Haroon, M. B.; Khursheed, A. B.; Shashi, P. and Abid, H. (2014). Antioxidant and cytotoxic activities of *Achillea millefolium* from Kashmir. J. of A. and Industrial Research, 2, 487-491.

Khalid, A. K. and Jaime, A. T. S. (2012). biology of *Calendula officinalis* Linn: focus on pharmacology biological activities and agronomic practices. Med. And Aromatic P. Sin And Bio. Tecnology, 12-27.

Mattila, P.; Astola, J. and Kumpulainen, J. (2000). Determination of flavonoids in plant material by HPLC with diode- array detections. J. Agric. Food Chem., 48: 5834-5841.

Muley, B. P.; Khadabadi, S. S. and Banarase, N, B. (2009). Phytochemical Constituents and Pharmacological Activities of *Calendula officinalis* Linn (Asteraceae). Tropical Journal of Pharmaceutical Research, 5, 455-465.

Munir, N.; Ayesha, S. I.; Imran, A.; Rasheeda, B.; Nadia, S.; Faiza, S. and Shagufta. N. (2014). Evaluation of antioxidant and antimicrobial potential of two endangered plant species *Atropa belladonna* and *Matricaria chamomilla*. Afr. J. Tradit Complement Altern Med, 5, 111-117.

Paşca, M. B.; Pallag, A and Gîtea, D. (2013). The quantitative determination of active principles from *Calendula officinalis* L. inflorescences. Uni. din Oradea, F. de Medicină Farmacie, Specializarea Farmacie, 317-320.

Preethi, K. C.; Girija, K. and Ramadasan, K. A. (2006). Antioxidant Potential of an Extract of *Calendula officinalis* Flowers in Vitro and in Vivo. Pharmaceutical Biology, 44, 691–697.

Rigane, G.; Ben-Younes, S.; Ghazghazi, H. and Ben-Salem, R. (2013). Investigation into the biological activities and chemical composition of *Calendula officinalis* L. growing in Tunisia. International Food Research Journal, 20, 3001-300.

Roopashree, T. S.; Raman, D.; Shobha, R. H. and Narendra, C. (2008). Antibacterial activity of antipsoriatic herbs: *Cassia tora, Momordica charantia* and *Calendula officinalis*. Int. J. of Applied Research in Natural Products, 3, 20-28.

Shan, B.; Caia, Y. Z.; Brooksb, J. D. and Corke, H. (2007). The in vitro antibacterial activity of dietary spices and medicinal herb extracts. International Journal of Food Microbiology, (112-119).

Vitalini, S.; Beretta, G.; Iriti, M.; Orsenigo, S.; Basilico, N.; Dall'Acqua, S.; Iorizzi, M. and Fico, G. (2011). Phenolic compounds from Achillea millefolium L. and their bioactivity. Biochimica Plonica 58, 203–209.