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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITIES OF SOME WILD MEDICINAL PLANTS OF THE WESTERN MEDITERRANEAN COASTAL REGION, EGYPT

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ABSTRACT: Four wild plant species (*Chrysanthemum cornarium*, *Capparis spinosa*, *Carthamus lanatus*, *Haplophyllum tuberculatum*) were investigated for their antioxidant activity, phenolic and flavonoid contents of their methanolic extracts. The antioxidant activity of the extracts was assessed their free radical scavenging properties using DPPH. Total phenolics and total flavonoids were detected by spectrophotometric and HPLC methods. The HPLC analysis compared with 14 reference standard phenolic compounds, results confirmed that gallic acid, chlorogenic acid, caffeic acid, 2,5- dihydroxy benzoic acid, and catechin were detected in the four methanolic plant extracts. HPLC chromatograms of the plant extracts showed that there was noticeable valuation in the distribution of phenolic compounds in the four studied species. Six bacterial strains were used in this study, three Gram-positive and three Gram-negative. Concerning with antimicrobial activity, results showed that plant extracts of the studied species are a great source of phenolic compounds and represent the highest antibacterial activity against Gram-positive and negative bacteria. Results revealed that extracts represents the highest antibacterial activity against Gram-positive and negative bacteria and largest inhibition zone for bacterial strains were exhibited by the extracts of *Carthamus lanatus* (11 mm) followed by that of *Haplophyllum tuberculatum* (8mm). However, *Chrysanthemum cornarium* extract showed the least inhibition zone (5mm).

INTRODUCTION: Nature has been the source of medicinal agents for thousands of years, and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. These plant based traditional medical systems continue to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care¹. There is great promise for new drug discovery based on traditional plant uses. There are multipurpose benefits from the natural flora or threatened plant species².

The grate surge of public interest in the use of plants as medicines has been based on the assumption that the plants will be available on a continuing basis. However, no concerted effort has been made to insure this, in the face of the threats posed by increasing demand, a vastly increasing human population and extensive destruction of plant-rich habitat such as tropical forests, wetlands, Mediterranean ecosystems and parts of arid zone. Today many medicinal plants face extinction or severe genetic loss, but detailed information is lacking. For most of the endangered medicinal plant species no conservation action has been taken.

Polyphenols are naturally occurring secondary metabolites in all plant materials, and prominently ubiquitous in herbs, vegetables, fruits, and seeds³. The most accruing polyphenols are flavonoids⁴. The other common polyphenols are phenolic acids. Polyphenols are chemopreventers, protecting the

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body tissues against oxidative stress and modulating gene expression and inhibiting UV and carcinogen induced tumorigenesis^{5, 6}. They also exhibit a wide range of biological activities including antimutagenic, antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic and vasodilatory actions⁷.

The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites which are synthesized and deposited in specific parts or in all parts of the plant. They may exert their action by resembling endogenous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters and thus have beneficial medicinal effects on humans due to similarities in their potential target sites. Therefore, random screening of plants for active chemicals is as important as the screening of ethnobotanically targeted species⁸.

Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective in preventing free radicals formation by scavenging them or promoting their decomposition and suppressing such disorders^{9, 10, 11}. There is an increasing interest in the measurement and use of plant antioxidants for scientific research¹². This is mainly due to their strong biological activity, exceeding those of many synthetic antioxidants

which have possible activity as promoters of carcinogenesis¹³.

The most prominent family of antioxidants from plants is represented by phenolic compounds. This fact is well reflected through the large number of plants containing phenolic compounds as dominant active principles¹⁴. Polyphenols constitute one of the most numerous and widely-distributed groups of substances in the Plant Kingdom, with more than 8000 phenolic structures currently known¹⁵. Among phenolic compounds, flavonoids represent the most common group¹⁴.

The objective of this study was to explore antioxidant activity, phenolic and flavonoid contents of methanolic extracts from 4 wild plant species restricted to the western Mediterranean region. These plants are threatened and need suggestion action for conservation, they are multipurpose species and in need for conservation program as they are natural resources for many ecosystem services (mainly medicinal & traditional uses). Analysis for their phytochemical components and evaluation of their antibacterial properties were also objectives to be achieved in this study.

MATERIALS AND METHODS:

Plant material

Plant material was air dried under shade, three samples of each plant species were taken for chemical analysis. Complete description of the studies plant species, their distribution, habitats and life forms are described in **Table 1**¹⁶.

TABLE 1: FULL DESCRIPTION OF THE PLANT SPECIES SELECTED FOR THE STUDY

Plant species	Distribution		Current Status	Main Habitat	Diagnostic Features
	National	Local			
<i>Glebionis coronaria</i> (L.) Tzveleu. (<i>Chrysanthemum</i> <i>cornarium</i>) (I)	Nd, Nv, Mma, Mp	31° 22' 27.00''N 27° 03' 46.50'' E	Threatened	Wadi mouth & barley fields	Glabrous annuals
<i>Capparis spinosa</i> L. var. <i>inermis</i> Turra. (II)	M.	31° 22' 26.40''N 27° 00' 19.98'' E	Threatened	Wadi bed, slope and maritime cliffs	Shrubs, stems ascending, woody climbers, with stipular spines.
<i>Carthamus lanatus</i> L.(III),	Nf, Mma Mma	31° 03' 41.16''N 28° 12'31.62'' E	Threatened	Non-saline depressions, wadis	Annual or biennial, stem straw colored, lanate- villous and glandular One of the summer flowering thistles.
<i>Haplophyllum</i> <i>tuberculatum</i> (Forssk.) Juss. (IV))	Nd, Nf, Nd	31° 23' .733''N 27° 01' .612'' E	Threatened	Road Sides, edges of cultivated lands and sandy soil	Perennial herbs, woody at the base, glabrous or crispate-hairy, with projecting glands on the stems and leaves.

Preparation of extracts

The plant materials were ground to a coarse powder. The powdered plant materials (each 10 g) were individually extracted with 100 ml of methanol kept on a rotary shaker for 24 h. Thereafter, it was filtered and centrifuged at 5000 g for 15 min. The supernatant collected and the solvent was evaporated to make the final volume one-fifth of the original volume. It was stored at 4 °C in airtight bottles for further studies.

Determination of Total Phenolics Content

The total phenolic content were determined with the Folin-Ciocalteu method¹⁷. The reaction mixture were contained 200 µl of plant extract, 800 µl of freshly prepared diluted Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The final mixture was diluted to 7 ml with deionized water. Mixtures were kept in dark at ambient conditions for 2 h to complete the reaction. The total phenolic content was expressed as mg gallic acid equivalents g⁻¹ dry sample.

Determination of Total Flavonoids Content

Total flavonoid content of the methanolic extracts was estimated according to Ismail *et al.*¹⁸. Total flavonoid content was determined using aluminium chloride (AlCl₃), and rutin (standard). The plant extract of 0.1 ml was added to 0.3 ml distilled water followed by 5% NaNO₂ (0.03 ml). Then, AlCl₃ (0.03 ml, 10%) was added, after 5 min and at 25°C. After further 5 min, the reaction mixture was treated with 0.2 ml of 1 mM NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. The results were expressed as mg rutin g⁻¹ dry sample.

Radical-Scavenging Activity—DPPH Assay

Radical scavenging activity of plant extracts against stable 2, 2 diphenyl 1 picryl hydrazylhydrate (DPPH) was determined by the method of Braca *et al.*¹⁹. DPPH reacts with an antioxidant compound, which can donate hydrogen, and reduce DPPH. The change in colour (from deep violet to light yellow) was measured at 517 nm on a UV visible light spectrophotometer (T80+ UV-Vis spectrometer, double beam). Briefly, 1 mL of aliquots of the extract and standards (20-100 µg mL⁻¹) was added to methanol solution of DPPH (5 mL, 0.1 mM) and vortexed. The samples were kept in the dark for 20 minutes at room temperature and the decrease in absorbance was measured at 517 nm against a blank. The results were expressed as

EC50, which means the concentration at which DPPH radicals were quenched by 50%. Radical scavenging activity was calculated by the following formula: % Inhibition = [(A_B - A_A)/A_B] × 100 Where A_B = absorption of blank sample, A_A = absorption of test extract solution.

HPLC analysis

High performance liquid chromatography was performed by using the analytical HPLC system. Twenty micro litter sample extract analyzed with a Exclipse XDB C₁₈ (5 µm, 4.6 X 150 mm) column using a mobile phase consisting 1 % (v/v) formic acid in aqueous solution:acetonitrile: 2- propanol (70:22:8) , pH 2.5 ; flow rate: 0.75 ml/ min, temperature : 30 °C , UV detection at 320 nm: Agilent technologies 1200 series. Identification and peak assignment of the compound was based on comparison of its retention time with corresponding standard and by spiking of sample with the standard. Quantification of the compound was done using total peak area and each peak with external standard.

Antibacterial activity

Six bacterial strains were used in this study, these bacteria consisted of three Gram-positive *Bacillus subtilis*, *Micrococcus luteus* and *Methicillin resistant Staphylococcus aureus* (MRSA) and three Gram-negative, *Acinetobacter baumannii*, *Klebsiella pneumonia*, and *Pseudomonas aeruginos*. The bacterial isolates were first subcultured in a nutrient broth (Oxoid) and incubated at 37°C for 18h.

Plant extracts were dissolved in 10% DMSO (Dimethyl sulfoxide) to a final concentration of 2 mg/mL, and the antibacterial activities of the methanolic extracts determined by Kirby-Bauer's disc diffusion method as per National Committee for Clinical Laboratory Standards (NCCLS) recommendations²⁰. Nutrient agar media plates were cultured with inoculums of each bacterium isolate using sterile cotton swabs. The discs of extracts placed on the sterile plates. The plates were incubated at 37°C for 24 hour. The average inhibition zone diameters (AVIZD) were measured and recorded.

Statistical analysis:

The results of all performed experiments were expressed as Mean ± SD of three determinations, the test of significance was applied wherever

necessary and values obtained as $p < 0.05$ were considered as statistically significant.

RESULTS:

The phytochemical analysis:

Phenolic and flavonoid contents are important in antioxidant power of herbal plants. Phenolic compounds content (**Figure 1**) presented in the examined extracts are calculated using gallic acid as a reference compound. The results of quantitative determinations of phenolic compounds obtained from the four plant species (*Chrysanthemum cornarium* (I), *Capparis spinosa* (II), *Carthamus lanatus* (III), *Haplophyllum tuberculatum* (IV)) are shown in **Figure 1**.

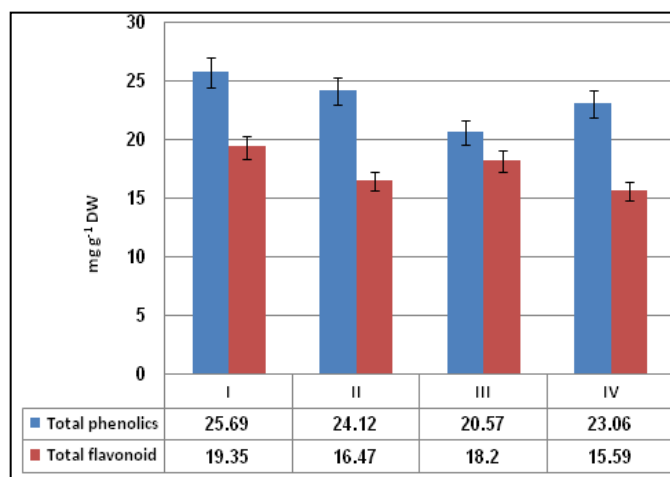


FIGURE 1: TOTAL PHENOLIC AND FLAVONOID CONTENTS OF FOUR PLANT SPECIES EXTRACTS

The highest values of phenolic compounds were shown in *Chrysanthemum cornarium* (I) 25.69 ± 0.614 , and *Capparis spinosa* (II) 24.12 ± 0.838 . Flavonoid content (Figure 1) presented in examined extracts are calculated using rutin as a reference compound. The flavonoids values were recorded, the richest species being *Chrysanthemum cornarium* (19.35 ± 0.481) and *Carthamus lanatus* (18.2 ± 0.287). HPLC chromatograms of analysed extracts are shown in **Figure 3**, and **Table 2**.

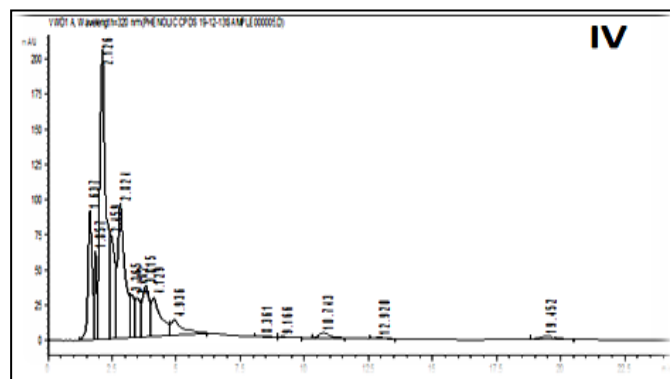
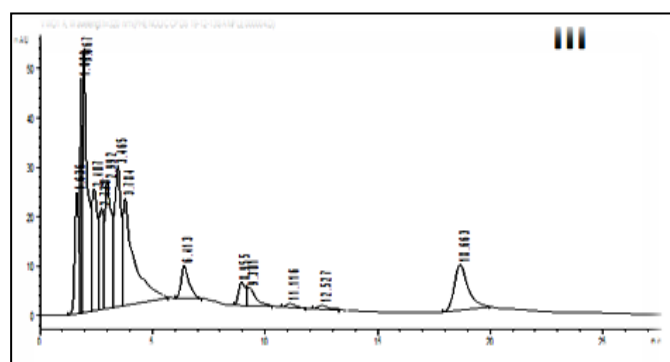
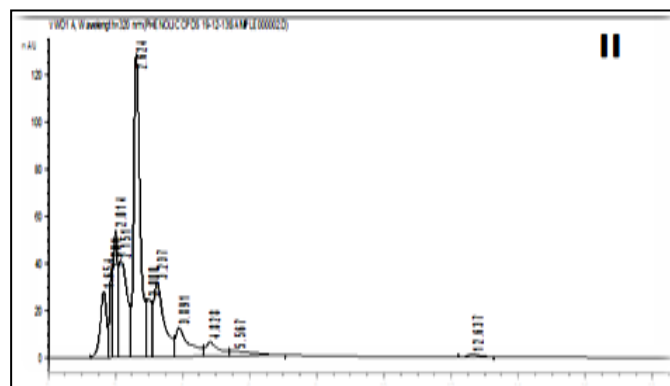
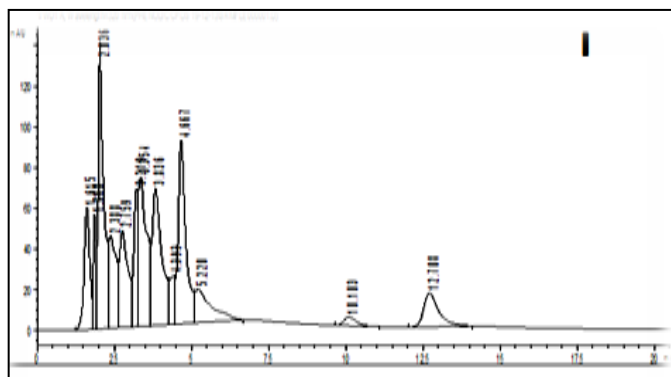


FIGURE 3: HPLC CHROMATOGRAM FOR METHANOLIC EXTRACTS OF FOUR PLANT SPECIES

Compared with 14 reference standard phenolic compounds, the HPLC- analysis results for the methanolic extracts of the studied plants showed that there was noticeable valuation in the distribution of different phenolic compounds in the four different species. 3, 5- Dicafeoyl quinic acid, Geraniol, Phloridzin, and Rutin, were not detected in *Chrysanthemum cornarium*. In *Capparis spinosa* 4, 5-Dicafeoyl quinic acid, Geraniol, and Quercetin were not detected in plant extract. While in *Carthamus lanatus*, 3, 4- Dicafeoyl quinic acid, Cinnamic acid, Phloridzin, and Quercetin were not detected in plant extract. In *Haplophyllum tuberculatum* 3, 5- Dicafeoyl quinic acid, Geraniol, and Rutin were not detected. Tannic acid was not detected in all methanolic extracts of the four studies plant species.

TABLE 2: HPLC ANALYSES OF PHENOLIC CONSTITUENTS OF THE METHANOLIC EXTRACTS OF PLANT SPECIES

Peak Name	RT (min)				Area MAU				Amt/Area %				Amount (mg ml ⁻¹)			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Chlorogenic acid	2.38	2.15	2.43	2.40	759.70	731.97	490.17	420.69	6.01	6.00	5.86	5.79	4.56	4.39	2.87	2.43
Caffeic acid	2.75	2.62	2.75	2.73	896.68	1710.13	1263.59	297.23	1.08	1.14	1.12	8.20	9.71	19.59	14.17	2.43
3,4- Dicafeoyl quinic acid	2.99	3.00	2.99	2.99	--	278.87	--	511.81	--	7.61	--	7.47	--	2.12	--	3.80
3,5- Dicafeoyl quinic acid	3.21	3.23	3.19	3.20	609.43	690.05	226.26	-	1.73	1.73	1.77	-	10.58	11.96	4.01	--
4,5-Dicafeoyl quinic acid	3.35	3.34	3.34	3.46	1391.99	--	244.27	556.00	7.31	--	8.94	7.83	10.17	--	2.18	4.35
2,5- dihydroxy Benzoic acid	3.83	3.89	3.97	3.78	148.75	347.13	495.32	761.79	2.40	4.80	3.86	3.09	3.56	1.66	1.91	2.35
Cinnamic acid	12.70	12.63	12.93	12.52	571.29	33.01	--	25.23	1.55	8.11	--	1.02	8.86	2.67	--	2.58
Geraniol	7.66	7.66	8.04	7.80	--	--	10.03	--	--	--	2.69	--	--	--	2.70	--
Gallic acid	1.86	1.88	1.83	1.82	330.58	186.15	274.80	242.45	8.45	8.22	8.39	8.34	2.79	1.53	2.30	2.02
Phloridzin	5.22	5.56	5.20	5.2	630.69	100.22	--	--	4.65	8.58	--	--	2.93	8.6	--	--
Quercetin	9.12	9.12	9.12	8.95	--	--	--	96.68	--	--	--	7.69	--	--	--	7.44
Catechin	3.83	3.89	3.97	3.78	1482.75	347.13	459.32	761.79	8.23	8.17	8.19	8.21	122.14	28.37	40.60	62.61
Rutin	4.66	4.82	4.73	4.80	1571.32	162.02	293.59	--	5.29	4.36	4.82	--	8.31	7.06	1.41	--
Tannic acid	5.90	5.90	5.90	5.90	--	--	--	--	--	--	--	--	--	--	--	--

Free Radicals Scavenging Activity

The results of the DPPH scavenging assay showed that all the extracts were able to reduce the stable DPPH radical to yellow-coloured diphenylpicrylhydrazine. The DPPH radical scavenging activity was recorded in terms of % Inhibition as shown in **Figure 2**.

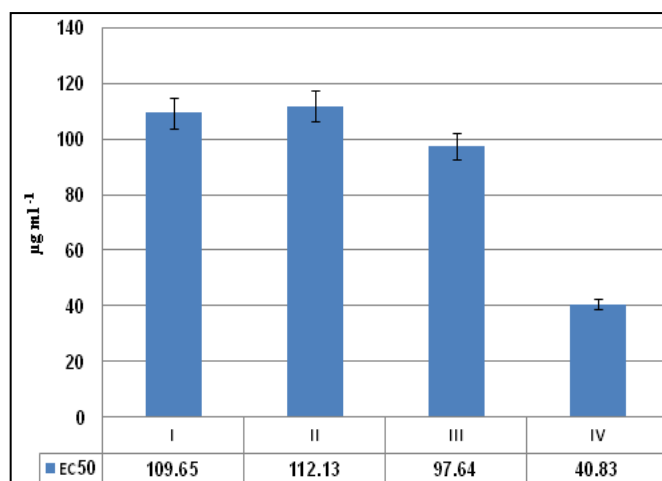


FIGURE 2: EC50 VALUES DPPH RADICAL SCAVENGING ACTIVITY OF FOUR PLANT SPECIES EXTRACTS.

It was observed that *Haplophyllum tuberculatum* has minimum DPPH scavenging activity (40.33 ± 2.03) and *Capparis spinosa* has maximum DPPH scavenging activity (112.13 ± 1.77) among plant

extracts. DPPH scavenging activity of *Chrysthanthemum cornarium* was 109.65 ± 0.56 , and *Carthamus lanatus* was 97.64 ± 1.25 .

Antimicrobial Activities

The antibacterial of the extracts obtained from methanolic extracts of the aerial parts the plants under study by the diffusion method are shown in **Table 3, Figure 4, Figure 5 and Figure 6**. Among the extracts tested, **I, II, III, and IV** were showed varying degree of antibacterial activities against the test bacterial species (**Table 3**). *K.pneumonia* showed the most resistance against the investigated extract AVIZD (0 mm) while *P.aeurginosa* exhibited the most sensitive AVIZD (17 mm). The order of the rest bacterial species was arranged as following: *A.bumanuiia* AVIZD (12 mm), *M.leutus* AVIZD (9 mm), *B. subtilis* AVIZD (7 mm) and MRSA AVIZD (2 mm).

Extract (**III**) the most active extract against investigated bacterial species AVIZD (11 mm), while extract (**I**) the lower activity AVIZD (5 mm), the other of the rest extract was arranged as following: (**IV**) AVIZD (8 mm) and (**II**) AVIZD (7 mm). Results showed that extracts are a great source of phenolic compounds and represents the highest antibacterial activity against Gram-positive and negative bacteria.

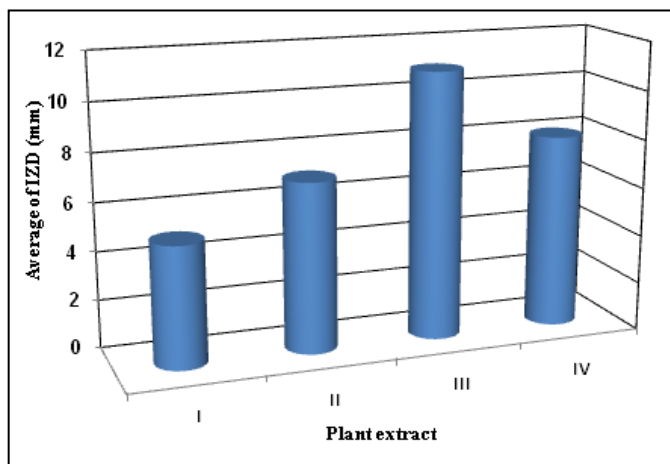


FIGURE 4: AVERAGE INHIBITION ZONE DIAMETER OF FOUR PLANT EXTRACTS AGAINST TESTED BACTERIA.

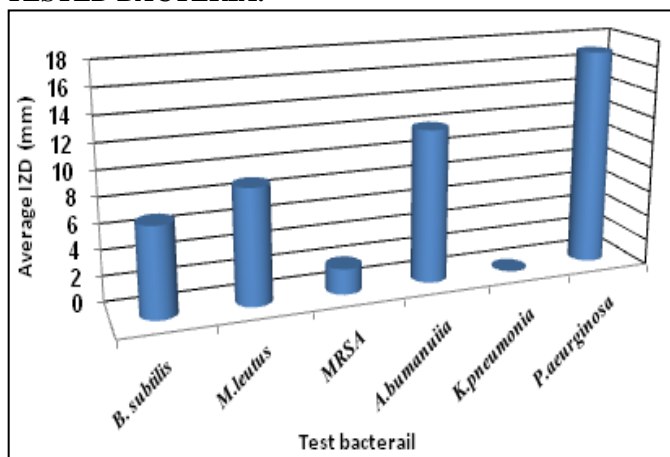


FIGURE 5: THE ANTIBACTERIAL ACTIVITY OF FOUR PLANT EXTRACTS AGAINST BACTERIAL SPECIES

TABLE 3: ANTIBACTERIAL ACTIVITY OF FOUR PLANT EXTRACTS

Bacterial Strain	IZD (mm)*				AV-extract
	I	II	III	IV	
<i>Bacillus subtilis</i>	6	0	10	11	7
<i>Micrococcus luteus</i>	11	0	18	6	9
MRSA	0	0	0	6	2
<i>Acinetobacter baumannii</i>	6	11	20	12	12
<i>Klebsiella pneumonia</i>	0	0	0	0	0
<i>Pseudomonas aeruginosa</i>	6	30	20	11	17
AV-bacterial	5	7	11	8	8

Inhibition Zone Diameter (mm)*

DISCUSSION: Numerous crude extracts and pure natural compounds from plants are reported to have antioxidant and radical-scavenging activities and intensive research has been carried out, either to characterize the antioxidant properties of extracts and/or to isolate and identify the compounds responsible for those activities seeking the development of natural antioxidant formulations in the areas of food, medicine and cosmetics^{21, 22, 23}.

The antioxidant activity of medicinal plants is mainly related to their phenolic compounds²⁴.

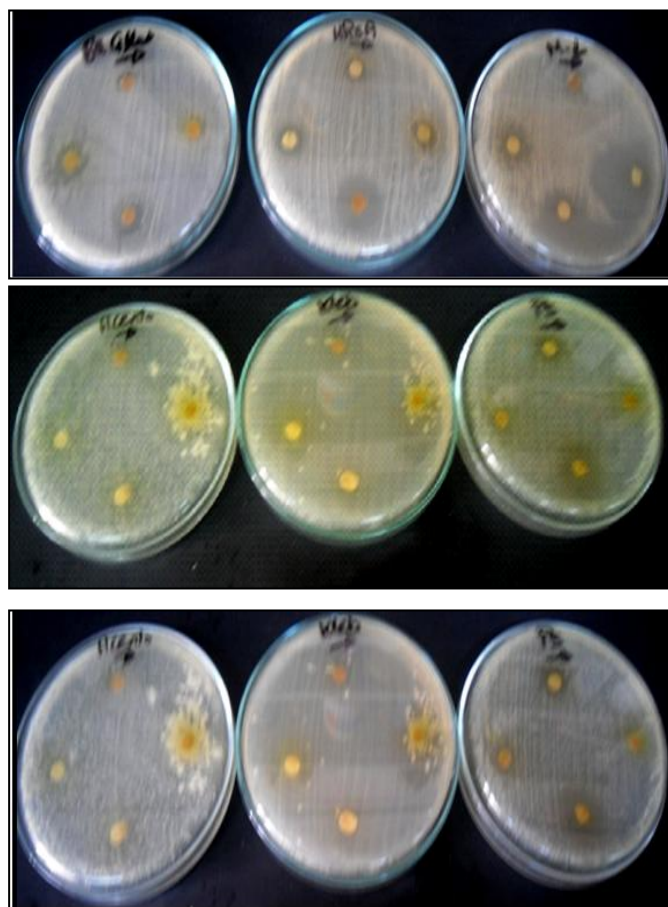


FIGURE 6: ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACTS OF FOUR PLANT SPECIES AGAINST MRSA, *B. SUBTILIS*, *ACINETOBACTER BAUMARRII*, *P. AEURGINOSA*, *KLEBSIELLA PNEUMONIA* AND *MICROCOCCUS LEUTUS* FOR 24 H

Polyphenol are the major plant compounds and are commonly found in both edible and inedible plants and they have been reported to have multiple biological effects, including antioxidant activity. Their antioxidant activity is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triple oxygen, or decomposing peroxides. The importance of the antioxidant constituents of plant materials in the

maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers and consumers²⁵. In the view of the up surging interest in the health benefits of the medicinal plants, we examined total phenolics, flavonoids and evaluated the antioxidant properties of four studied plants (*Chrysthanthemum cornarium* (I), *Capparis spinosa* (II) *Carthamus lanatus* (III), *Haplophyllum tuberculatum*(IV)

DPPH is a stable nitrogen-centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers²⁶. It was found that the DPPH scavenging activity of four plant species are varied in the values of DPPH scavenging activity, *Haplophyllum tuberculatum* < *Carthamus lanatus* < *Capparis spinosa* < *Chrysthanthemum cornarium* that has maximum (112.13±1.77) among plant extracts. The EC50 was calculated, and expressed as the amount of antioxidant exists in the sample necessary to decrease the initial DPPH concentration by 50%. The DPPH scavenging capacity of the plant extracts may be related to the phenolic compounds present²⁷.

DPPH radical was used as a stable free radical to determined antioxidant activity of natural compounds²⁸. It is reported that the decrease in the absorbance of DPPH radical caused by phenolic compounds is due to the reaction between antioxidant molecules and radicals, resulting in the scavenging of the radical by hydrogen donation and is visualized as a discoloration from purple to yellow²⁹.

The phytochemical analysis of the combination of four selected medicinal plants they all are rich in total phenolic compounds, flavonoids. Accordingly; these compounds have shown to have antioxidant activity³⁰. Total phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators hence it was reasonable to detect their amount in the herbal preparation. Flavonoids are the most widespread group of natural compounds and probably the most important natural phenolics. Total phenolics and flavonoids possess a broad spectrum of chemical and biological activities

including radical scavenging properties. Such property is especially distinct for flavonols.

The medicinal effects of plants are often attributed to the antioxidant activity of phytochemical constituents mainly phenolics, flavonoids and flavonols. It is claimed that phenolic compounds are powerful chain breaking antioxidants. The scavenging activity of phenolic group is due to its hydroxyl group³¹. Reactive oxygen species [ROS] cause oxidative damage to the tissues and protection from such damages are provided by endogenous and exogenous antioxidants. Plant based antioxidants are preferred due to the multiple mechanisms of actions and of the phytochemicals present in them³². Kaur and Mondal³³ reported that in order to realize the health benefits from potential plant sources, it is important to measure the antioxidant activity using various radicals and oxidation systems.

The antibacterial activity of plant extracts can be attributed not only to a single bioactive principle but also in concert action with other compounds³⁴. The results indicated that the methanolic extracts of all the species studied showed antibacterial activities towards the Gram-positive and- negative bacteria. These results are consistent with previous reports on related plants regarding Gram-positive bacteria³⁵. The resistance of Gram-negative bacteria (*Klebsiella pneumonia*) to plant extracts was not unexpected as; in general, this class of bacteria is more resistant than Gram-positive bacteria. Such resistance could be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism³⁶.

Results revealed that extracts represents the highest antibacterial activity against Gram-positive and negative bacteria and largest inhibition zone for bacterial strains were exhibited by the extracts of *Carthamus lanatus* (11mm) followed by that of *Haplophyllum tuberculatum* (8mm). However, *Chrysthanthemum cornarium* extract showed the least inhibition zone (5mm). The antibacterial activity of plant extracts can be attributed not only to a single bioactive principle but also in concert action with other compounds³⁴. The chemical structure of the antimicrobial agents found in higher plants belong to most commonly encountered classes of higher plants secondary metabolites such as flavonoids³⁷, and phenolic acids³⁸. Plant extracts are great sources of phenolic

compounds and represent the highest antibacterial activities against Gram-positive bacteria³⁹. Long term observation of the studies species and because of multipurpose uses of these plants they are currently threatened and need a conservation program, their habitat are fragmented. *Chrysanthemum cornarium*, for example showed the highest phenolic and flavonoid contents.

CONCLUSION: It can be summarized that, folk herbal medicines are important source of drug discovery as they are widely used for the control of plant pathogens and human related diseases. The medicinal effects of plants are often attributed to the antioxidant activity of phytochemical constituents mainly phenolics, and flavonoids. The four wild plant species hold important position in traditional system of medicines and their antimicrobial activities have been proved in the present study. Due to the increasing interest in the measurement and use of plant antioxidants for scientific research, this paper high lights the role of bioactive components and natural resources of plants under the study as a source of drugs with fewer side effect . Accordingly we aim at finding a financial support for a conservation program for this natural threatened resource and to enhance the developed of natural drug industry.

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