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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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DPPH Scavenging assay

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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 extract of the studied species are a great source of phenolic compounds and represents the highest antibacterial activity against Gram-positive and negative bacteria. Results revealed that extracts represent the highest antibacterial activity against Gram-positive and negative bacteria and largest inhibition zone for bacterial strains were exhibited by the extracts of *Haplophyllum tuberculatum* (79 mm) followed by that of *Capparis spinosa* (54mm). However, *Chrysanthemum cornarium* extract showed the least inhibition zone (34 mm).

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 care ¹. There is great promise for new drug discovery based on traditional plant uses.

There are multipurpose benefits from the natural flora or threatened plant species ². 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 continuingly. However, no concerted effort has been made to ensure this, in the face of the threats posed by increasing demand, a vastly increasing human population and extensive destruction of plant-rich habitats such as tropical forests, wetlands, Mediterranean ecosystems and parts of the 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 seeds ³.

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The most accruing polyphenols are flavonoids⁴. The other common polyphenols are phenolic acids. Polyphenols are chemoprevention, protecting the 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 all parts of the plant. They may exert their action by resembling endogenous metabolites, ligands, hormones, signal transduction molecules of 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 a 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 of 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 the shade, three samples of each plant species were taken for chemical analysis. A 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 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 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. After that, 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 contents were determined with the Folin-Ciocalteu method¹⁷. The reaction mixture was contained 200 µl of diluted rice bran 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 the 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 aluminum 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 a 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.

DPPH Radical Scavenging Activity Assay: Radical scavenging activity of plant extracts against stable 2, 2-diphenyl 2-picrylhydrazyl hydrate (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 color (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 the methanol solution of DPPH (5 mL, 0.1 mM) and vortexed. The samples were kept in the dark

for 20 min 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:

$$\% \text{ Inhibition} = [(A_B - A_A) / A_B] \times 100$$

Where A_B = absorption of the 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 liter sample extract analyzed with a Eclipse XDB C₁₈ (5 µm, 4.6 × 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 the corresponding standard and by spiking of the sample with the standard. Quantification of the compound was done using total peak area and each peak with an 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 pneumoniae*, and *Pseudomonas aeruginosa*. The bacterial isolates were first subcultured in a nutrient broth (Oxoid) and incubated at 37 °C for 18 h.

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 isolated using sterile cotton swabs. The discs of extracts placed on the sterile plates. The plates were incubated at 37 °C for 24 h. The average

inhibition zone diameters (AVIZD) were measured and recorded.

Statistical Analysis: The results of all performed experiments were expressed as Mean \pm 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 **Fig. 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 **Fig. 1**.

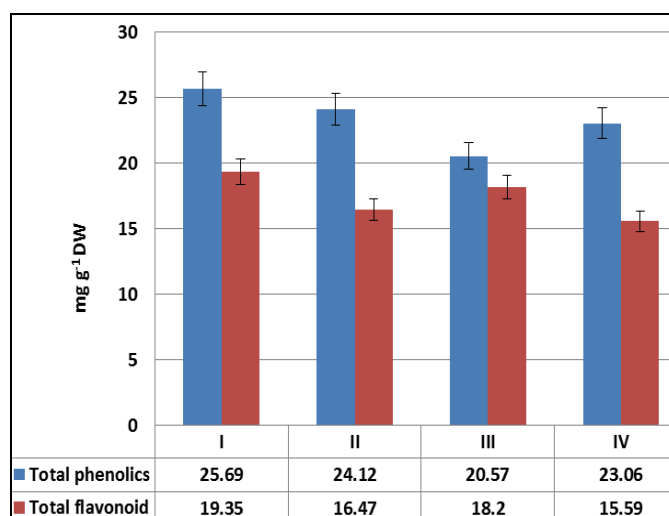


FIG. 1: TOTAL PHENOLIC AND FLAVONOID CONTENTS OF FOUR PLANT SPECIES EXTRACTS

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

Peak name	RT (min)				Area (MAU)			
	I	II	III	IV	I	II	III	IV
Chlorogenic acid	2.38	2.15	2.43	2.40	759.70	731.97	49.017	420.69
Caffeic acid	2.75	2.62	2.75	2.73	896.68	1710.13	1263.59	297.23
3,4-Dicaffeoyl quinic acid	2.99	3.00	2.99	2.799	-	278.87	-	511.81
3,5-Dicaffeoyl quinic acid	3.21	3.23	3.19	3.20	609.43	690.05	226.26	-
4,5-Dicaffeoyl quinic acid	3.35	3.34	3.34	3.46	1391.99	-	244.27	556.00
2,5-dihydroxy benzoic acid	3.83	3.89	3.97	3.78	148.45	347.13	495.32	761.79
Cinnamic acid	12.70	12.63	12.93	12.52	571.29	33.01	-	25.23
Geraniol	7.66	7.66	8.04	7.80	-	-	10.03	-
Gallic acid	1.86	1.88	1.83	1.82	330.58	186.15	274.80	242.45
phloridzin	5.22	5.56	5.20	5.2	630.69	100.22	-	-
Quercetin	9.12	9.12	9.12	8.95	-	-	-	96.68
Catechin	3.83	3.89	3.97	3.78	1482.75	347.13	459.32	761.79
Rutin	4.66	4.82	4.73	4.80	1571.32	162.02	293.59	-
Tannic acid	5.90	5.90	5.90	5.90	-	-	-	-
Peak name	Amount / Area (%)				Amount (mgml ⁻¹)			
	I	II	III	IV	I	II	III	IV
Chlorogenic acid	6.01	6.00	5.86	5.79	4.56	4.39	2.87	2.43
Caffeic acid	1.08	1.14	1.12	8.20	9.71	19.59	14.17	2.43
3,4-Dicaffeoyl quinic acid	-	7.61	-	7.47	-	2.12	-	3.80
3,5-Dicaffeoyl quinic acid	1.73	1.73	1.77	-	1058	11.96	4.01	-
4,5-Dicaffeoyl quinic acid	7.31	-	8.94	7.83	10.17	-	2.18	4.35
2,5-dihydroxy benzoic acid	2.40	4.80	3.86	3.09	3.56	1.66	1.91	2.35
Cinnamic acid	1.55	8.11	-	1.02	8.86	2.67	-	2.58
Geraniol	-	-	2.69	-	-	-	2.70	-
Gallic acid	6.01	6.00	5.86	5.79	4.56	4.39	2.87	2.43
phloridzin	1.08	1.14	1.12	8.20	9.71	19.59	14.17	2.43
Quercetin	-	7.61	-	7.47	-	2.12	-	3.80
Catechin	1.73	1.73	1.77	-	1058	11.96	4.01	-
Rutin	7.31	-	8.94	7.83	10.17	-	2.18	4.35
Tannic acid	2.40	4.80	3.86	3.09	3.56	1.66	1.91	2.35

The highest values of phenolic compounds were shown in *Chrysanthemum cornarium* (I) $25.69 \pm$

0.614 and *Capparis spinosa* (II) 24.12 ± 0.838 . Flavonoid content **Fig. 1** presented in examined

extracts are calculated using rutin as a reference compound. The flavonoids values were recorded, the richest species being *Chrysthanthemum cornarium* (19.35 ± 0.481) and *Carthamus lanatus* (18.2 ± 0.287).

HPLC chromatograms of analyzed extracts are shown in **Fig. 3** and **Table 2**. Compared with 14 reference standard phenolic compounds, the HPLC-analysis results for the methanolic extracts of the studied plants showed that there was noticeable evaluation in the distribution of different phenolic compounds in the four different species. 3, 5-Dicaffeoyl quinic acid, Geraniol, Phloridzin, and Rutin, were not detected in *Chrysthanthemum cornarium*.

In *Capparis spinosa* 4, 5-Dicaffeoyl quinic acid, Geraniol, and Quercetin were not detected in the plant extract. While in *Carthamus lanatus*, 3, 4-Dicaffeoyl quinic acid, Cinnamic acid, Phloridzin, and Quercetin were not detected in the plant extract. In *Haplophyllum tuberculatum* 3,5-Dicaffeoyl quinic acid, Geraniol, and Rutin were not detected. Tannic acid was not detected in all methanolic extracts of the four studies plant species.

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-colored diphenylpicrylhydrazine. The DPPH radical scavenging activity was recorded in terms of % Inhibition, as shown in **Fig. 2**.

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 .

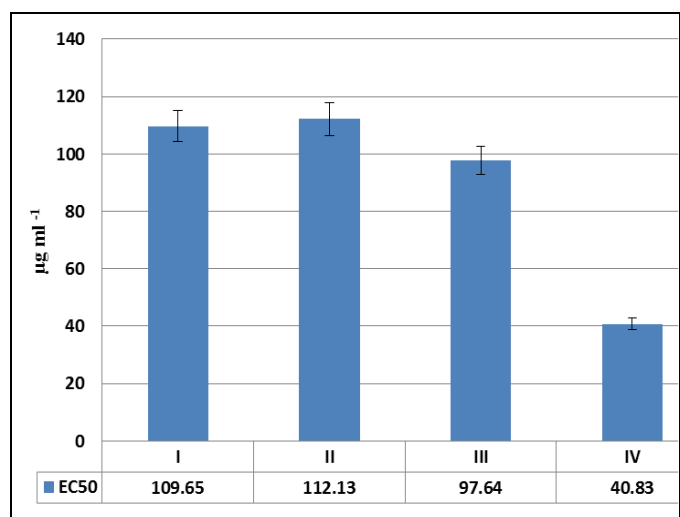
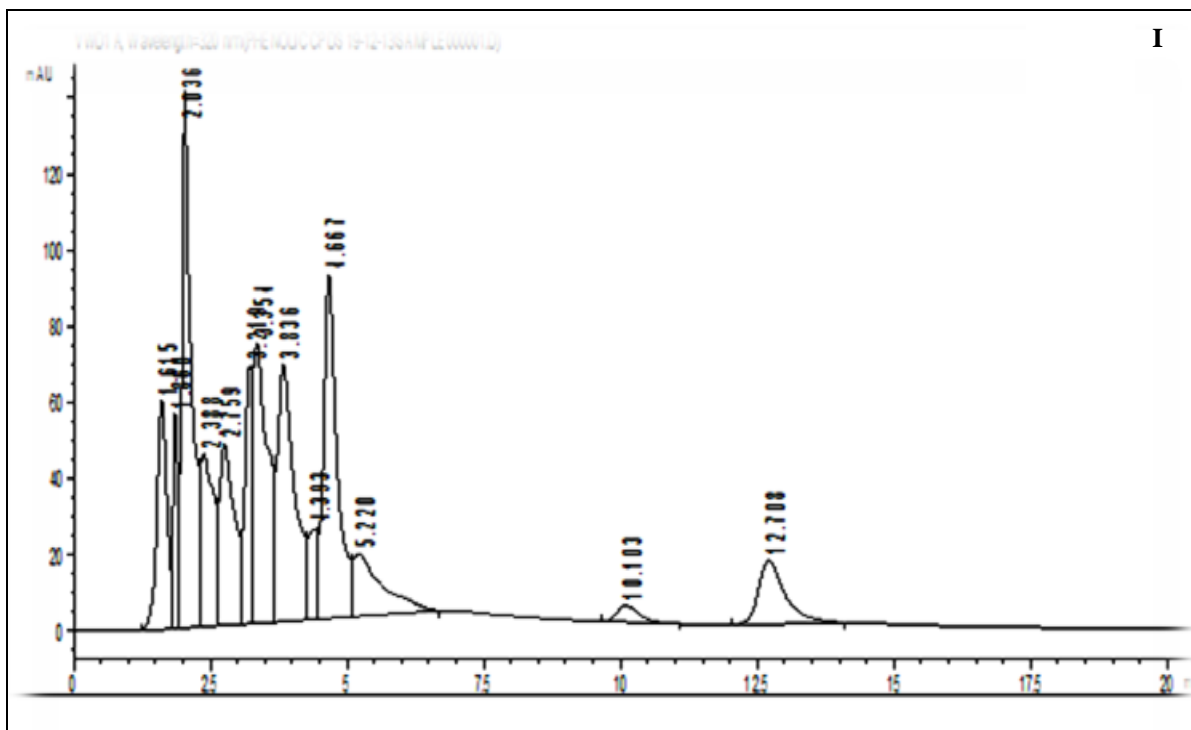


FIG. 2: EC₅₀ VALUES DPPH RADICAL SCAVENGING ACTIVITY OF FOUR PLANT SPECIES EXTRACTS



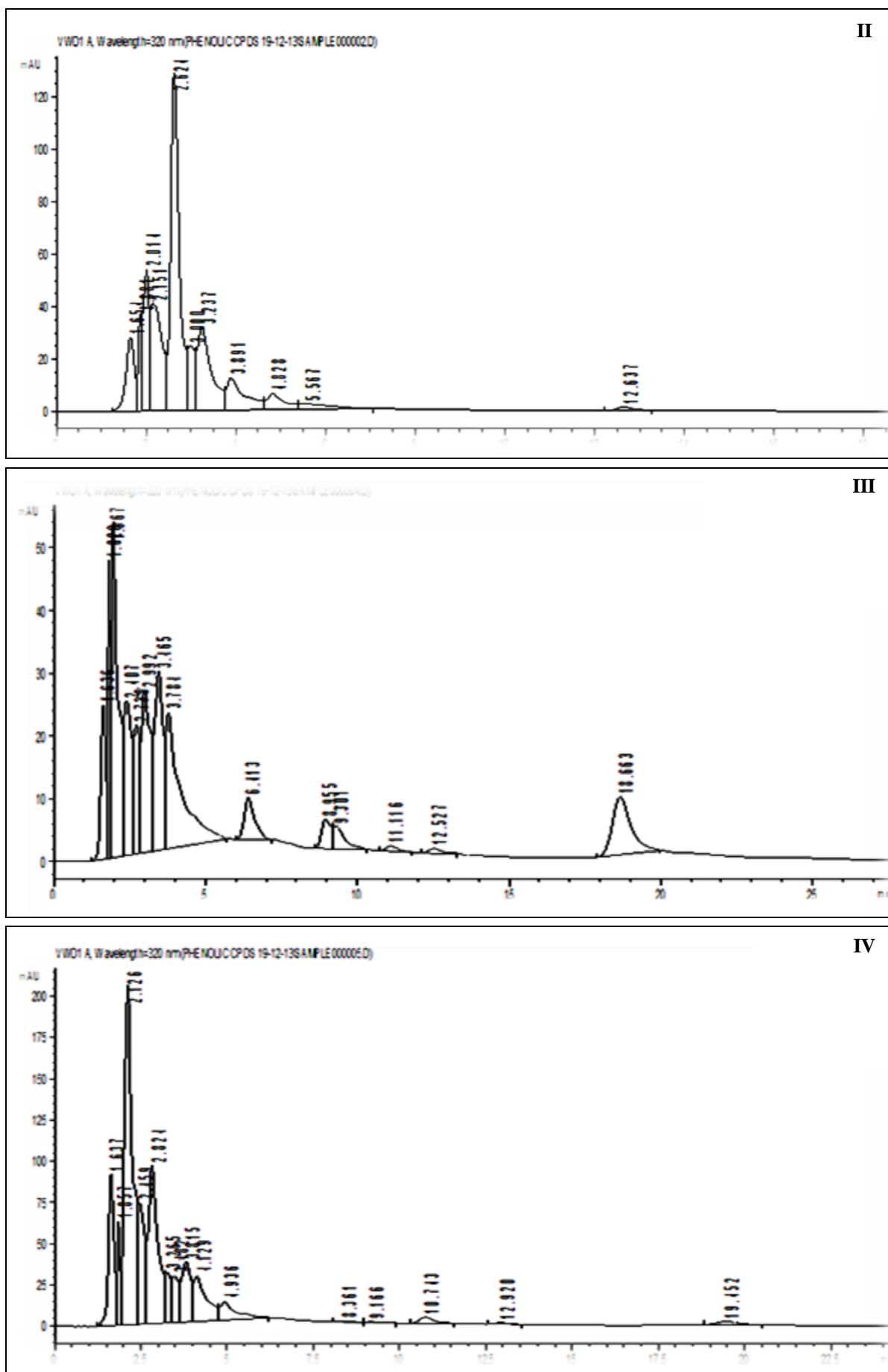


FIG. 3: HPLC CHROMATOGRAM FOR METHANOLIC EXTRACTS OF FOUR PLANT SPECIES

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**, **Fig. 4**, **Fig. 5**, and

Fig. 6. Among the extracts tested, **I**, **II**, **III**, and **IV** were showed varying degree of antibacterial activities against the test bacterial species **Table 3**.

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)*

K. pneumonia showed the most resistance against the investigated extract AVIZD (0 mm) while *P.aeruginosa* exhibited the most sensitive AVIZD (17 mm). The order of the rest bacterial species were arranged as following: *A.bumanuua* 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 follows: (IV) AVIZD (8 mm) and (II) AVIZD (7 mm). Results showed that extracts are a great source of phenolic compounds and represent the highest antibacterial activity against Gram-positive and negative bacteria.

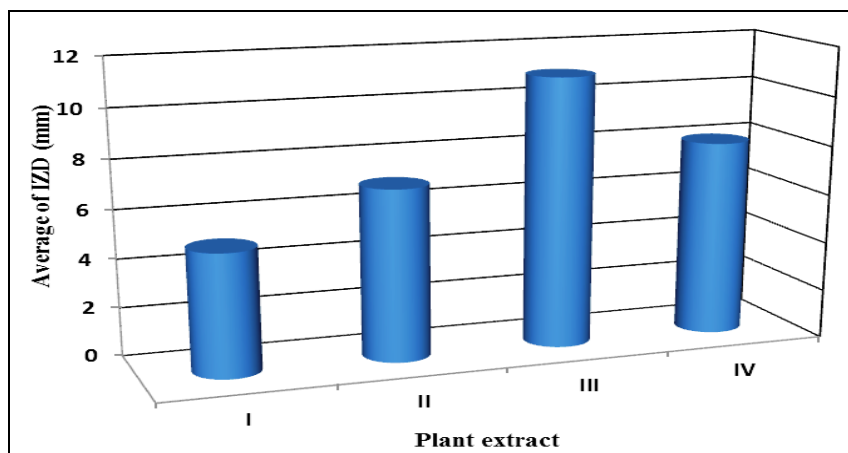


FIG. 4: AVERAGE INHIBITION ZONE DIAMETER OF FOUR PLANT EXTRACTS AGAINST TESTED BACTERIA

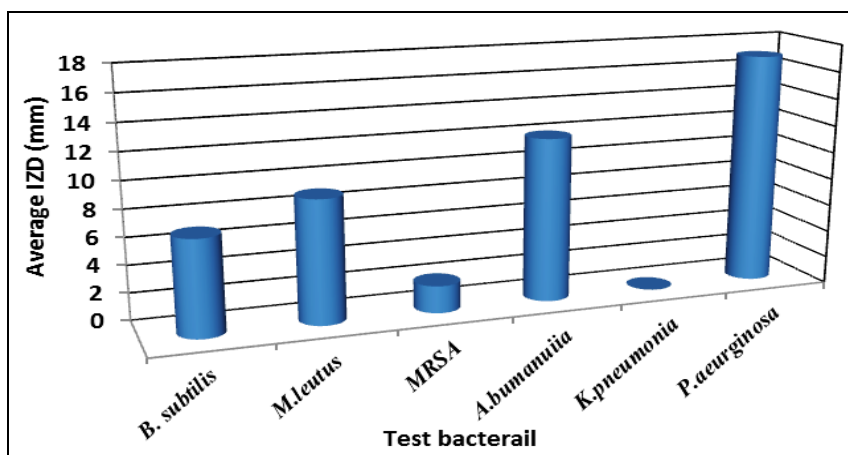


FIG. 5: THE ANTIBACTERIAL ACTIVITY OF FOUR PLANT EXTRACTS AGAINST BACTERIAL SPECIES

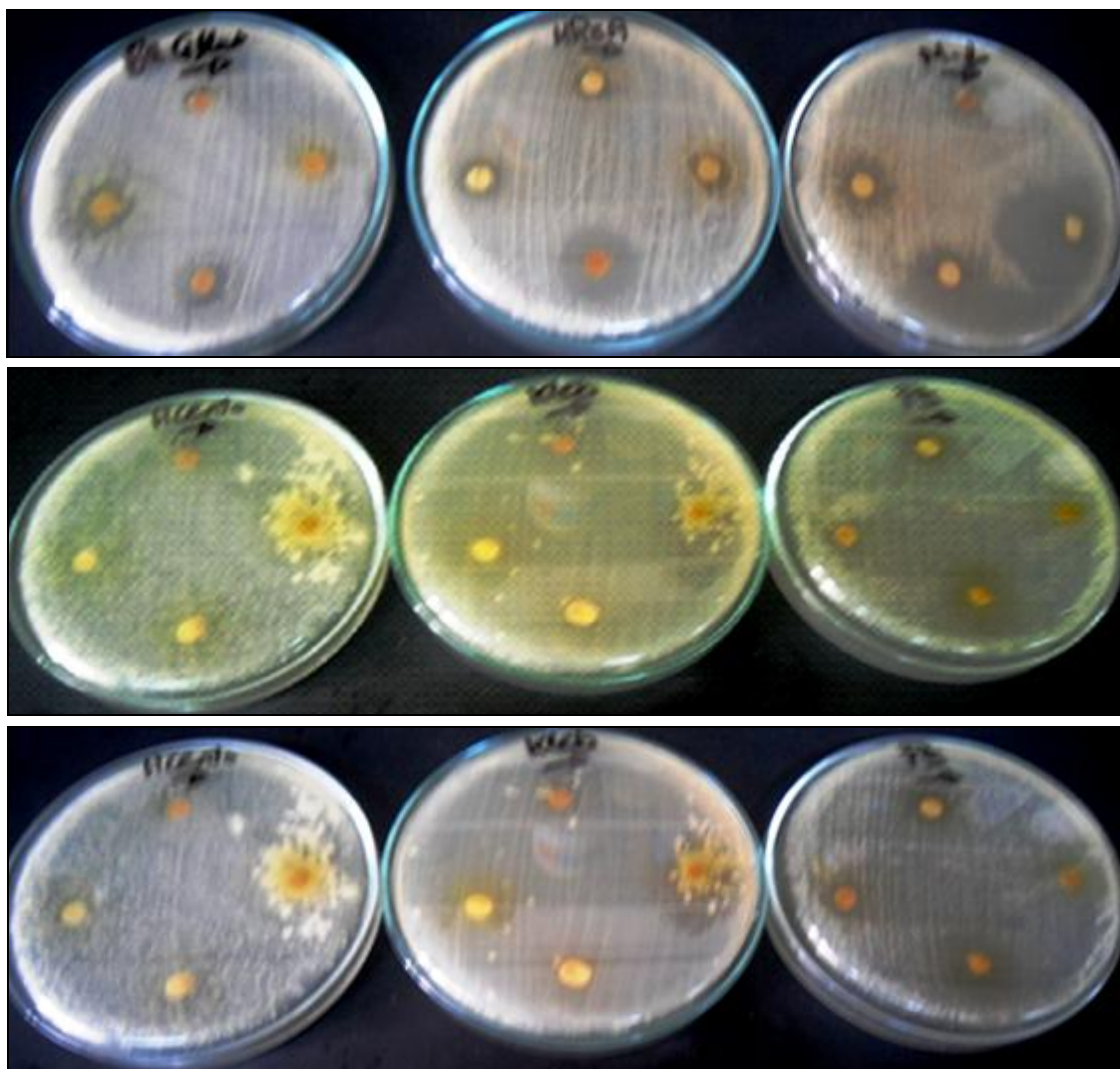


FIG. 6: ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACTS OF FOUR PLANT SPECIES AGAINST MRSA, B. SUBTILIS, ACENITOBACTER BAUMARRII, P. AEURGINOSA, KLEBSIELLA PNEUMONIA AND MICROCOCCUS LEUTUS FOR 24 H

DISCUSSION: Numerous crude extracts and purely 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. Polyphenol is the major plant compounds and is 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 absorbing and neutralizing free radicals, quenching singlet, and triplet 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 upsurging interest in the health benefits of the medicinal plants, we examined total phenolics, flavonoids and evaluated the antioxidant properties of four studied plants (*Chrysanthemum 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 can perform this reaction can be considered as antioxidants and therefore radical scavengers²⁶. It was found that the DPPH scavenging activity four plant species are varied in the values of DPPH scavenging activity, *Haplophyllum tuberculatum* < *Carthamus lanatus* < *Capparis spinosa* < *Chrysothamnium 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 determine the 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 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 the phenolic group is due to its hydroxyl group³¹. Endogenous and exogenous antioxidants provide reactive oxygen species [ROS] cause oxidative damage to the tissues and protection from such damages.

Plant-based antioxidants are preferred due to the multiple mechanisms of actions and the phytochemicals present in them³². Kaur and Mondal³³ reported that 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 represent the highest antibacterial activity against Gram-positive and negative bacteria and largest inhibition zone for bacterial strains were exhibited by the extracts of *Haplophyllum tuberculatum* (79mm) followed by that of *Capparis spinosa* (54mm). However, *Chrysothamnium cornarium* extract showed the least inhibition zone (34 mm).

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 the 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 is fragmented. *Chrysanthemum cornarium*, for example, showed the highest phenolic and flavonoid contents.

CONCLUSION: It can be summarized that folk herbal medicines are an 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 an important position in the 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 a fewer side effect. Accordingly, we aim at finding financial support for a conservation program for this natural threatened resource and to enhance the developed of the natural drug industry.

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CONFLICT OF INTEREST: Nil

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