

Original Research

LC-ESI-MS/MS Profiling, Phytoconstituent Evaluation, Antimicrobial and Antioxidant Effect of Selected 8 Medicinal Plants Growing in Saudi Arabia

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Received: 15 May 2025

Accepted: 08 November 2025

Abstract

Medicinal plants are the primary source of resources for practically all traditional healthcare systems. A single herb or mixtures of multiple plants thought to have complementary and/or synergistic effects can be found in herbal medications used in traditional medicine. The aim of this investigation is to identify the bioactive components in the ethanol extracts of eight medicinal plants collected from wild populations in three valleys in the Medina region, Saudi Arabia (Wadi Al-Baidha, Al-Aqiq, and Al-Fora'a). The plants, namely, *Asphodelus fistulosus*, *Commicarpus grandiflorus*, *Heliotropium arbainense*, *Pulicaria incise*, *Rhazya stricta*, *Rumex vesicarius*, *Senna alexandrina*, and *Withania somnifera*, were analyzed using Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-Mass). The ethanol extract demonstrates the presence of bioactive phenolic compounds, the main components of which were quercetin, luteolin, quercetin, kaempferol-3-glucuronide, and naringenin. The ethanolic extract and its subsequent fractions' total phenolics and antioxidant activity were quantified. Ethanolic extract had the largest percentage of total phenolic for *R. stricta* (134 mg/gdw), while *R. vesicarius* had the highest percentage of total antioxidant (97%). Well-diffusion techniques were used to examine the antimicrobial potential. The findings showed that *A. graveolens* exhibited significant antibacterial activity against three kinds of bacteria: *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*. Based on the present results, it is also possible to extend the exams in order to utilize them for medicinal applications.

Key words: LC-ESI-MS/MS, herbal plants, antioxidant, total phenolic, and antimicrobial activity

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Introduction

In developing nations with long histories of traditional medical systems, the first primary healthcare facility utilizing traditional medicine was founded in 1926. Later, the growing tendency of folk medicine was positively impacted by the biomedical hospitals established in the latter part of the 20th century. Because of their biological advantages, *Plectranthus* species are used in traditional medicine in Saudi Arabia's primary healthcare system. Some plant species are well-known for their biological properties as antiseptics, wound healers, and remedies for intestinal, stomach, liver, heart, and respiratory conditions [1].

According to Tonisi et al. [2], it is well known that several plants in the genus *Plectranthus* possess a high capacity for biosynthesis, forming a variety of phytochemical classes from cell secondary metabolism, primarily diterpenes, phenolics, and triterpenoids. A sizable portion of the Arabian Peninsula is covered by the arid landmass of Saudi Arabia, which has a geologic structure [3].

Saudi Arabia is blessed with a diverse array of plants, including several trees, shrubs, and medicinal herbs. Before the advent of biomedicine, folk medicine was widely practiced in Saudi Arabia and is regarded as an integral part of the country's cultural legacy [4]. There are over 2,250 plant species in Saudi Arabia [5]. However, there are approximately 44 families, 125 genera, and 184 species in the flora of the Makkah district. According to Ayoub et al. [6], a significant portion of these species, 24.57%, are used medicinally. It is characterized by a number of ecosystems with diverse plant species, including xerophytic vegetation, medicinal plants, and important genetic resources for crop plants [7]. To accurately characterize medicinal plants and show their phenolic component composition, numerous sensitive and selective analytical techniques have been developed [8].

Numerous cancers, diabetes, degenerative disorders, etc., are mostly caused by free radicals [9]. The absence of a natural antioxidant defense system results in the production of these free radicals [10]. Almost all plant species contain flavonoids [11]. The free radical scavenging activity of natural antioxidant agents is used to treat many diseases, such as Alzheimer's disease, diabetes, stroke, atherosclerosis, and cancer [4].

The phenolic components of medicinal plants, including flavonoids, lignans, catechins, and anthocyanins, may be responsible for their antioxidant properties [12]. Flavonoids and phenolics are potent antioxidants that strengthen the immune system, according to recent research. According to Neupane and Lamichhane [13], a diet high in flavonoids can protect against cardiovascular, neurological, and cancerous conditions. According to numerous researches, women who consume more flavonoids through their diet are less likely to acquire breast cancer [14].

Therefore, this study aims to extract and investigate phytochemical analysis of ethanolic plant extract in Saudi Arabia to determine their main constituents using (LC-ESI-MS/MS) of each individual plant extract using comparative analysis with some phenolic, antioxidant, and antimicrobial activity.

Materials and Methods

Plant Collections

During the winter, eight plant species were gathered from various locations, including three valleys in the Medina region (Wadi Al-Baidha, Al-Aqiq, and Al-Fora'a), as shown in Fig. 1. They were then preserved in plastic bags until the required analysis was completed [15].

Plants Identification and Preparation of Samples

Plants were gathered, dried, and placed in Jeddah University's herbarium. According to Saudi Arabian flora, the eight plants (*Asphodelus fistulosus*, *Commicarpus grandiflorus*, *Heliotropium arbainense*, *Pulicaria incise*, *Rhazya stricta*, *Rumex vesicarius*, *Senna alexandrina*, and *Withania somnifera*) depicted in Fig. 2 were identified and verified using the Chaudhary plant protection method [16].

Preparation of the Extract

Following the initial filtration, the dried plant was placed in a flask with approximately 1:15 of 95% ethanol, and the resulting ethanolic extract was stored in the refrigerator. Do the same thing twice. After that, samples were heated in a water bath beneath a condenser to heat the extract. After a day, the extracts were repeatedly filtered through filter paper and stored in the dark at 4°C until they were needed [17].

Performance Characteristics of the LC-ESI-MS/MS Method

Liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) with an Exion LC AC system for separation and a SCIEX Triple Quad 5500+ MS/MS system with an electrospray ionization (ESI) system for detection were used to analyze the plant extracts [18].

Total Polyphenol Content (TPC)

The Folin-Ciocalteu method was used to measure the TPC levels in ethanol plant extract in triplicate at 765 nm [19]. The calibration curve was created using gallic acid (GA). Gallic acid equivalents per 100 g dry weight (mg GAE/100 g DW) were used to express the TP contents.

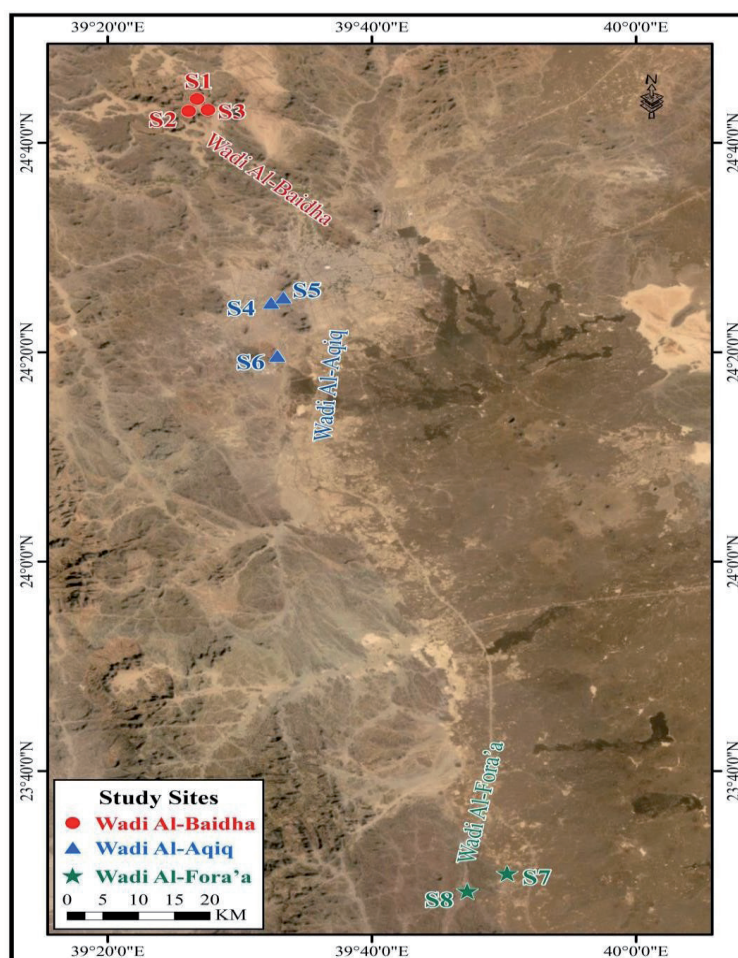


Fig. 1. Map of the Study Area in three valleys (Wadi Al-Baidha, Al-Aqiq, Al-Fora'a) in the Medina region [5].

Determination of DPPH Free-Radical Scavenging Activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay was carried out by the method of Mekni spectrophotometrically [20]. Aliquots (50 μ l) of various plant extracts were added to 5 ml of a 0.004% ethanol solution of DPPH. After incubating the samples for 30 min at room temperature, the absorbance was read against a blank at 517 nm. Ascorbic acid (AA) was used as a reference standard and dissolved in methanol to make the stock solution with the same concentration (1 mg/mL).

$$I(\%) = (1 - AS/AC) \times 100 \quad (1)$$

Where AC is the absorbance of the control reaction (containing all reagents except the tested compound), and AS is the absorbance of the tested compound. The % of inhibition was determined from a graph plotting percentage inhibition against extract concentration. All experiments were performed in duplicate.

Antimicrobial Activities

Tests for Antibacterial Activity

Gram-negative microbes *Escherichia coli*, ATCC 25922, 27853, and *Pseudomonas aeruginosa* NRRL B23 were also used, in addition to the two gram-positive bacteria *Bacillus subtilis* NRRL-B-4219 and *Staphylococcus aureus* (ATCC 25923). In order to compare the antibacterial activity of plant extract against human pathogenic bacteria, the well diffusion method [21] was used to complete the antibacterial tests. As a guide, neomycin was used at a concentration of 30 μ g/ml. Saline was added to the bacterial suspensions until they reached a concentration of 10^5 CFU/ml. To verify that there was no tainting and to verify the inoculum's authenticity, it was refined on nutrient medium. After spreading the societies onto the plates, a cork borer (8 mm) was used to create wells. Dimethyl sulfoxide (DMSO)-weakened designed mixtures with 10 μ g/ml were placed in well stacks, and foci (10 μ g/ml) were added to each well independently. Neomycin was utilized as guidelines, with a focus of 30 μ g/ml used as a standard antibacterial, and DMSO was used as a negative control. The Petri dishes were kept aseptically

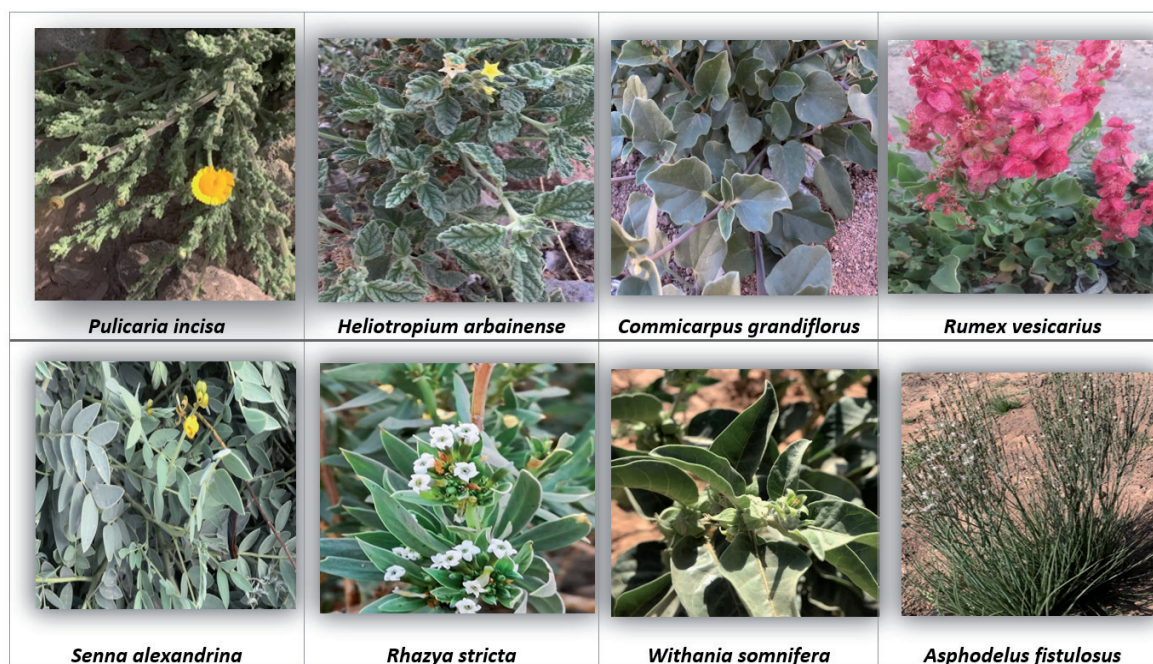


Fig. 2. Pictures and scientific names of the plant study. Photos by mobile iPhone 7 Model: A1778.

for roughly 4 to 5 h for dissemination of the example. Following, all the Petri dishes were incubated for 24 h at 32°C, then the development of inhibition zones was estimated.

Antifungal Activity

The potato dextrose agar medium was utilized for determining the antifungal activity of the plant extract. The medium was arranged and sanitized in an autoclave for 15 min at 15 psi. At that point, it was aseptically moved into the serialized Petri plates. After a term of 2 h., the contagious strains, yeasts, for example, *Candida albicans* ATCC 10231, and growths (*Aspergillus niger* NRRL-3 and *Penicillium sp.*), were immunized on the surface of Petri plates independently. The parasitic suspensions were balanced with clean saline solution to 10^4 spores/ml [22]. The inocula were refined on strong medium to confirm the nonappearance of pollution and to check the legitimacy of the inoculum. Societies were spread onto the plates, and after that, wells were made by utilizing a cork borer (8 mm). Wells were stacked with: 10 µg/ml of complex weakened with dimethyl sulfoxide (DMSO) at (50 µg/ml) were added to each well independently. These Petri plates were incubated for 48 h at a temperature of $28 \pm 2^\circ\text{C}$, and after that, the development of inhibition zones (in mm) was recorded. The zone of restraint (mm) of each compound was resolved and contrasted, and the standard medication Tetracycline antifungal activity was essentially estimated as subjective, and after that, the best outcomes were rehashed with focus (30 µg/ml).

Results and Discussion

In the current study, eight plants cultivated in Saudi Arabia were explored and characterized quickly using (LC-ESI-MS/MS) chromatographic analysis. Furthermore, the presence of certain flavonoids and phenolic acids was investigated in comparison to standard samples. International recognition of HPLC as a trustworthy technique for identifying herbs and herbal products is well established. In a complicated herbal system, it can identify both unknown and marker elements [17].

According to Table 1, Table 2 and Fig. 3, each plant sample contains a unique phenolic and flavonoid, all of the plants included the following compounds: coumaric acid, luteolin, syringic acid, ferulic acid, naringenin, vanillin, rutin, caffeic acid, chlorogenic acid, and 3,4-dihydroxybenzoic acid.

This chemical is important in the perfume industry and also gives the samples a unique aromatic smell depending on concentration [23]. Coumaric acid, which is typically found in fruit peels, has anti-inflammatory properties and helps keep fruits from going bad [17]. As an antioxidant, anti-allergic, anti-helminthic, and typically used astringent, rutin has numerous applications in leather tanning and dyeing [24]. Its molecules help lower cholesterol and control blood glucose levels, making it an anti-diabetic [25]. It contains antioxidant properties [26], helps dilate blood vessels, and guards against liver disorders, cancer, and cell damage [27].

The HPLC chromatograms in Fig. 3 showed a close similarity with minor caffeic acids in all plants under investigation. All of the plants under study had

Chromatogram of sample 11 showing peaks at retention times: 1.462, 6.024, 6.174, 6.289, 6.905, 10.274, 10.387, 11.702, 12.029, 12.827, 15.837, and 16.043. The x-axis is labeled 'Time' and the y-axis is labeled 'Intensity'.

Mass Spectrum (m/z vs. Relative Intensity):

m/z	Relative Intensity (approx.)
1.727	0.05
6.251	0.05
6.989	0.15
7.265	1.00
8.989	0.10
9.798	0.05
9.988	0.45
10.168	0.05

[illegible]

Chromatogram of the sample showing peaks at retention times 1.247, 1.379, and 1.470. The x-axis is labeled 'Time' and the y-axis is labeled 'Intensity'.

File: K2000017.D\\Data\\MS\\Scan 161

Mass Spectrum Plot (Scan 161) showing relative intensity (0.000 to 1.000) versus m/z (1 to 25). The base peak is at m/z 70. Other labeled peaks include m/z 43, 55, 57, 69, and 81.

Fig. 3. HPLC-ESI-MS/MS profile of stander and plant extracts.

a close resemblance to minor caffeic acids, according to the HPLC chromatograms in Fig. 3. Similar findings were consistent with numerous other *Plectranthus taxonomic* species from which similar acids have previously been found. In agreement with many other species of *Plectranthus* taxa from which these acids were previously identified [28]. Also, the LC-ESI-MS/MS analysis of *C. grandifloras* extract was in agreement with Mahato and Kundu, with isolation of two new triterpenes and seven known compounds [29]. There are differences between the eight species, which showed the presence or absence of apigenin, kaempferol, methyl gallate, quercetin, daidzein, and gallic acid. Cinnamic acid, catechin, myricetin, hesperetin, and ellagic acid were not detected in all studied plant samples.

Although we are mostly interested in the proteins, lipids, carbohydrates/sugars, and other vitamins that are present in food plants, we are also searching for compounds that have therapeutic value that are present in medicinal plants. These chemicals are commonly referred to as secondary metabolites and are not particularly necessary for plants' or organisms' normal growth and development. These substances are produced by plants in order to defend themselves against harmful organisms, illnesses, and environmental factors. Polyphenols, alkaloids, glycosides, terpenes, flavonoids, coumarins, tannins, and other SMs are primarily beneficial in medicine [12].

According to Sahar et al. [15], secondary metabolites made by plants, including phenolic compounds, have a wide range of therapeutic benefits, including anti-inflammatory, antibacterial, antiviral, and strong antioxidant qualities. From the above findings, Gallic acid may be used as a helpful tool for the chemotaxonomy of *Plectranthus taxa* that are grown in Saudi Arabia, we can conclude.

There is not many research on plant diversity in this area, despite the fact that its geographical makeup gives it a unique richness. However, there is no information about their exploration because no ethnobotany surveys have been attempted. Because of this, the flora of the Aljumu region has not gotten enough attention when it comes to documenting its wild medicinal plants. Therefore, the goal of this study was to record the medicinal plants that grow in the Aljumu region by conducting field surveys inside the study area.

Finally, HPLC is considered to be the principal analytical technique for quantitative analysis within complex matrices and qualitative determination of the targeted compounds.

Total Polyphenolic

The TPC values for all samples are shown in Fig. 4. Concerning total phenolics (mg GAEs/Kg D.W.) in different plant extracts. In this regard, there were quite notable differences among all the plants that were examined. Flavonoids and phenols were significant biologically active components because they were

thought to be antibacterial, antioxidant, and anticancer agents. etc., [30].

As shown in Fig. 4, it was found that *Rhazya stricta*, *Rumex vesicarius*, *Pulicaria incise*, and *Withania somnifera* were the most abundant (134, 77, 75, and 71), followed by *Heliotropium arbainense* and *Commicarpus grandiflorus* (65 and 57), while *Asphodelus fistulosus* and *Senna alexandrina* were found to be the least abundant in this regard (40 and 22).

There was a similarity between our results and those reported in the literature; the literature showed that these species were regarded as antioxidants. The chemical makeup of several of these extracts was examined, paying particular attention to flavonoids and anthraquinones in the case of *R. patientia* roots [31].

Flavonoids serve a variety of purposes in plants, and it has been proposed that they serve as secondary antioxidants defined in response to different types of stress. *Senna alexandrina* ethanol extracts exhibited higher TFC than the other extracts. The findings of this study are not consistent with those found in Mwamatope's literature. *Senna alexandrina*'s antioxidant activity results are comparable to those described in the literature, and there may be similar causes causing the differences in TPC [12].

Antioxidant Activity

Because of its stability in the radical state and ease of assay, the DPPH radical is a favored substrate for quickly evaluating antioxidant activity. Recent studies suggest that the antioxidant activity of phenolic compounds derived from plants, especially flavonoids, may be caused by -OH groups, which have redox characteristics [21].

The DPPH assay was used to evaluate the investigated plant extracts' capacity to scavenge free radicals in relation to ascorbic acid as standards. There was a discernible variance in the overall antioxidant activity of plant extracts from various plants (Fig. 5). The variance was somewhat consistent with the flavonoid and phenolic content. According to our research, the plant extracts are abundant in phenolic and flavonoid components, which are crucial for the body as dietary supplements and medications. According to numerous studies, these nutrients can support immunity, protect the liver, regulate elevated cholesterol, manage blood pressure, and more [32].

Rumex vesicarius' current antioxidant activity results were consistent with Demirezer et al. [33]. Antioxidant activity, total phenolics, and flavonoids results agreed that plant maturity and genotype may have contributed to the variation in phenolic compound concentrations [34].

The antioxidant activity was calculated as percentages. According to the data, the species of *H. zeylanicum* exhibited the most pronounced antioxidant activity, with a percentage of 92.79%. Similarly, a high antioxidant activity of around 84.08 was found

Table 1. RT of different studies on plants by LC-ESI-MS/MS.

Compound	Formula	RT	Concentration (µg/ml)							
			<i>A. fistulosus</i>	<i>C. grandiflorus</i>	<i>H. arbainense</i>	<i>P. incisa</i>	<i>R. stricta</i>	<i>R. vesicarius</i>	<i>S. alexandrina</i>	<i>W. somnifera</i>
Chlorogenic acid 355.1/163	$C_{16}H_{18}O_9$	7.34	17.22	1.38	6.17	101.66	0.13	1.34	0.72	5.61
Daidzein 255.1/199	$C_{15}H_{10}O_4$	12.92	0.01	0.00	0.00	0.02	ND	0.00	0.00	ND
Gallic acid 168.9/124.9	$C_7H_6O_5$	3.85	0.02	0.02	ND	ND	0.16	0.29	0.02	0.03
Caffeic acid 178/135	$C_9H_8O_4$	8.04	1.43	0.48	0.04	1.61	0.28	8.13	0.11	0.52
Rutin 609/299.9	$C_{27}H_{30}O_{16}$	9.71	3.91	29.84	0.05	0.64	0.76	23.23	3.75	21.33
Coumaric acid 162.9/119	$C_9H_8O_3$	9.53	0.27	0.80	0.42	0.24	0.18	0.54	0.14	0.67
Vanillin 151/136	$C_8H_8O_3$	9.57	0.17	0.09	0.17	0.32	0.10	0.16	0.11	0.29
Naringenin 271/119	$C_{15}H_{12}O_5$	15.04	7.71	2.19	0.63	13.55	0.15	1.50	2.12	2.10
Quercetin 301/151	$C_{15}H_{10}O_7$	13.58	0.01	0.00	ND	0.01	0.01	0.02	0.00	ND
Ellagic acid 301/145	$C_{14}H_6O_8$	9.92	ND	ND	ND	ND	ND	ND	ND	ND
3,4-Dihydroxybenzoic acid 152.9/109	$C_7H_6O_4$	5.72	0.38	4.55	1.35	5.91	0.78	0.70	0.21	1.26
Hesperetin 301/136	$C_{16}H_{14}O_6$	15.63	ND	ND	ND	ND	ND	ND	ND	ND
Myricetin 317/137	$C_{15}H_{10}O_8$	11.71	ND	ND	ND	ND	ND	ND	ND	ND
Cinnamic acid 146.9/102.6	$C_9H_8O_2$	14.19	ND	ND	ND	ND	ND	ND	ND	ND
Methyl gallate 183/124	$C_8H_8O_5$	7.45	ND	0.00	ND	0.00	0.00	ND	ND	0.00
Kaempferol 284.7/93	$C_{15}H_{10}O_6$	15.35	ND	ND	ND	0.03	0.01	0.01	0.01	ND
Ferulic acid 192.8/133.9	$C_{10}H_{10}O_4$	10.24	0.96	0.51	0.34	0.15	0.36	1.91	0.30	1.51
Syringic acid 196.8/181.9	$C_9H_{10}O_5$	8.4	0.64	0.08	0.08	0.48	0.15	0.23	0.08	0.17
Apigenin 269/151	$C_{15}H_{10}O_5$	15.05	1.16	ND	ND	ND	ND	0.21	0.33	ND
Catechin 288.8/244.9	$C_{15}H_{14}O_6$	7.34	ND	ND	ND	ND	ND	ND	ND	ND
Luteolin 284.7/132.9	$C_{15}H_{10}O_6$	13.52	23.45	0.01	0.01	0.06	0.02	0.18	4.19	0.01

Table 2. Area of different studies plants by LC-ESI-MS/MS.

Compound	Area									
	STD	A. fistulosus	C. grandiflorus	H. arbainense	P. incisa	R. stricta	R. vesicarius	S. alexandrina	W. somnifera	
Chlorogenic acid 355.1/163	2948	3172	25360	1136	1873000	23.13	246.8	133.5	1034	
Daidzein 255.1/199	6922	0.8439	125.2	1.164	694.3	ND	1.143	0.8433	ND	
Gallic acid 168.9/124.9	3293	3.106	469	ND	ND	32.62	58.74	4.308	6.516	
Caffeic acid 178/135	309.4	2759	92620	80.76	3118	539.5	15730	209.7	1000	
Rutin 609/299.9	201.5	4920	37580	57.46	804.5	953.8	29260	4728	26860	
Coumaric acid 162.9/119	532.8	902.2	2661	13.89	791.7	615	1785	469.3	2226	
Vanillin 151/136	17409	19.05	9452	18.13	34.96	10.9	17.29	12.03	31.31	
Naringenin 271/119	17.85	85.96	24.38	7.044	151.2	1.627	16.79	23.69	23.48	
Quercetin 301/151	296	11.14	2.613	ND	16.73	13.25	41.27	6.461	ND	
Ellagic acid 301/145	6.633	ND	ND	ND	ND	ND	ND	ND	ND	
3,4-Dihydroxybenzoic acid 152.9/109	25.53	59.94	725.7	215.3	943.8	123.9	111.9	33.5	200.7	
Hesperetin 301/136	62.72	ND	ND	ND	ND	ND	ND	ND	ND	
Myricetin 317/137	38.86	ND	ND	ND	ND	ND	ND	ND	ND	
Cinnamic acid 146.9/102.6	2.361	ND	ND	ND	ND	ND	ND	ND	ND	
Methyl gallate 183/124	491.3	ND	0.66	ND	0.7285	1.636	ND	ND	0.3826	
Kaempferol 284.7/93	26.2	ND	ND	ND	4.428	1.259	2.116	1.764	ND	
Ferulic acid 192.8/133.9	19.65	117.6	62.13	41.78	17.89	44.37	234.6	37.01	186	
Syringic acid 196.8/181.9	5.841	23.42	2.88	3.028	17.55	5.508	8.241	2.863	6.314	
Apigenin 269/151	1.456	10.57	ND	ND	ND	ND	1.934	2.99	ND	
Catechin 288.8/244.9	10.31	ND	ND	ND	ND	ND	ND	ND	ND	
Luteolin 284.7/132.9	159.5	23380	8.983	12.49	59.71	18.44	174.9	4173	6.757	

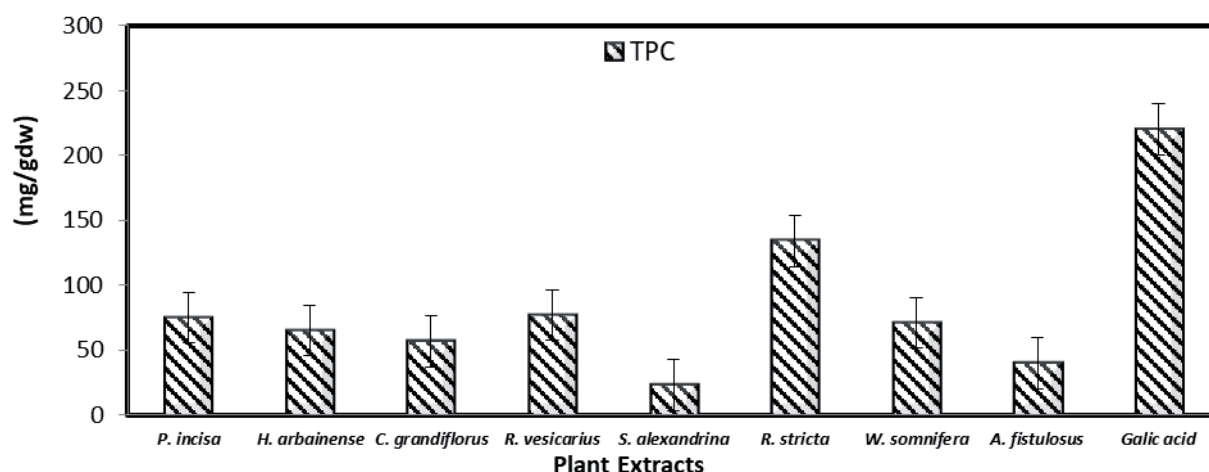


Fig. 4. Total polyphenolic contents of plant extracts.

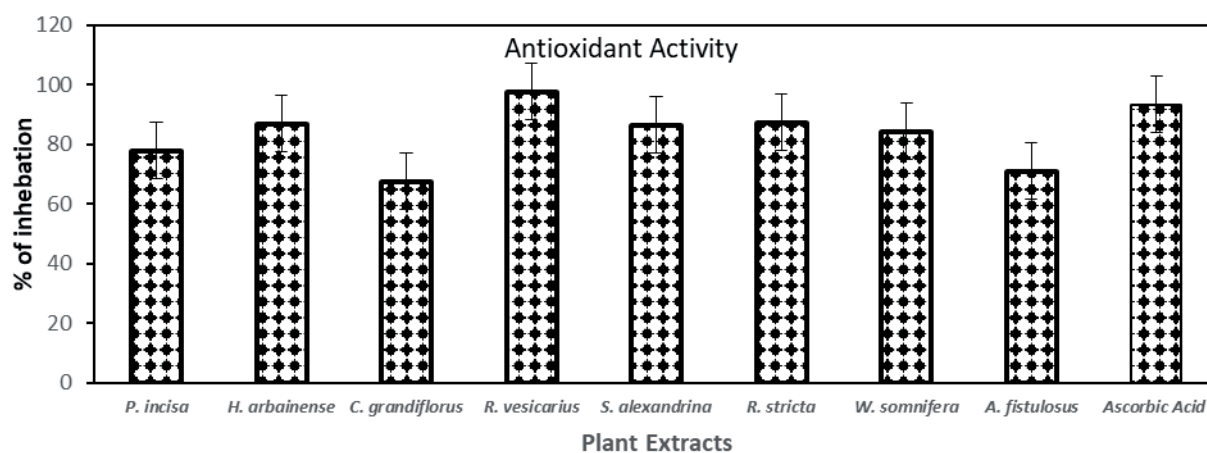


Fig. 5. Antioxidant activity of plant extracts.

by the species *H. bacciferum*. A clear difference was seen when the antioxidant activity of extracts from the *Heliotropium* species under study was measured [35].

Based on the findings, it appears that the antioxidant activity was directly correlated with the amount of phenolics present. To put it another way, the antioxidant activity increased with the phenolic concentration. In earlier studies, total antioxidant potential was utilized to compare species of cherry [36]. The levels of bioactive compounds that accumulate in plants may be directly impacted by different habitats. Abd-ElGawad et al. [37] examined the antioxidant activity and phenolic content of several plants gathered from Saudi Arabia. Results on antioxidant activity and total phenolics were consistent with those of El-Hawary et al. [38], who found that the presence of flavonoids and phenolics in *R. vesicarius* had hepatoprotective and antioxidant effects. The findings of *R. vesicarius* L.'s antioxidant activity, total phenolics, and flavonoids also concurred with those of Tavares et al. [39], who discovered that *R. maderensis*'s flavonoids and polyphenolics were highly

correlated with its antioxidant capacity, that the plant's antioxidant capacity is reflected in its total flavonoid and phenolic content, and that flavonoids are antioxidant molecules that scavenge free radicals with their concurrent oxidation.

Antimicrobial Activity

Plant extracts were evaluated for their antimicrobial activities against *S. aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans*, *Aspergillus niger*, and *Penicillium*. In the present study, the well diffusion observations reveal that ethanolic plant extracts were active against the three fungal strains *Candida albicans*, *Aspergillus niger*, and *Penicillium*, whereas the ethanolic plant extracts showed moderate activity, which was comparable to the standard drug tetracycline at a concentration of 30 µg/well. The *R. vesicarius*, *H. arbainense*, and *P. incisa* exhibited weaker antifungal activity against *Candida albicans* (22, 20, and 16 mm, respectively), compared to Tetracycline (22 mm). Moreover, only weak antibacterial activities

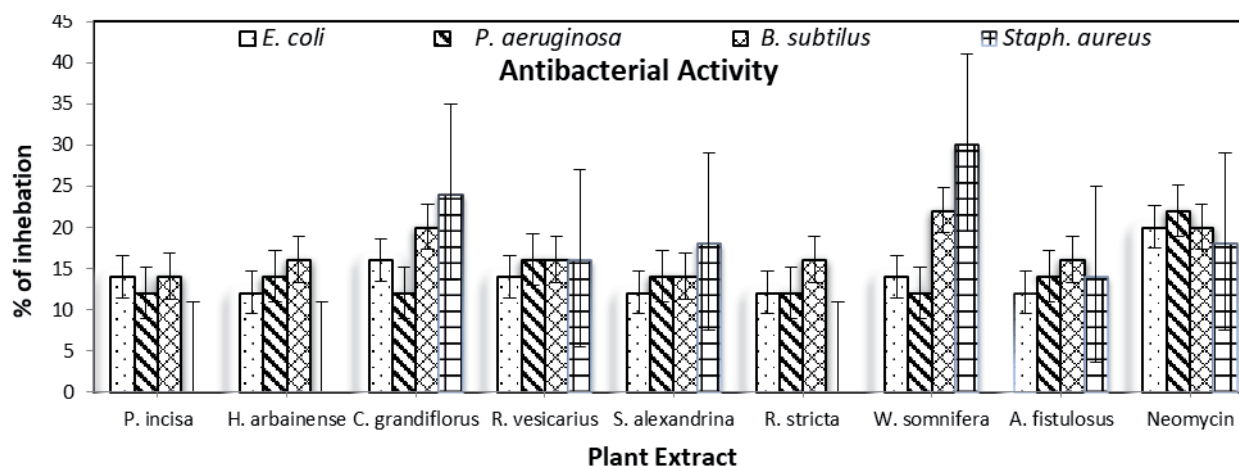


Fig. 6. Antibacterial activity of the plant extracts.

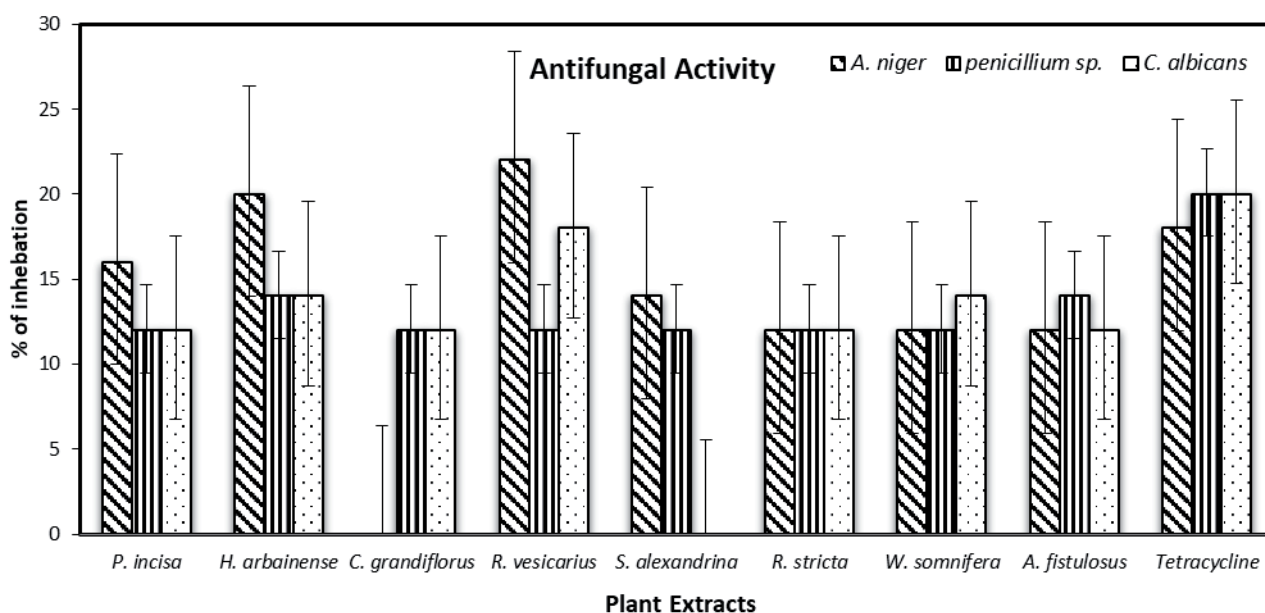


Fig. 7. The antifungal activity of plant extracts.

were observed towards all examined strains, and none of the samples inhibited the growth of the plant extracts. Numerous illnesses, including rheumatoid arthritis, cancer, Parkinson's and Alzheimer's diseases, are likely to occur as a result of the body producing oxygen and free radicals [40]

From the results shown in Figs 6 and 7, it was observed that Gram-positive bacterial strains were sensitive towards the various extracts. The zone of inhibition for *C. grandiflora*, *S. alexandrina*, and *W. somnifera* (24, 20, and 30 mm, respectively) against *Staph. aureus* compared with neomycin (18 mm). A minimum inhibitory concentration (MIC) value of > 64 mg/mL for eucalyptin (8 mg/ml) against *Staphylococcus aureus* was reported by Brezani et al. [41]. Shehabeldine et al. showed that this compound significantly damaged the cell walls and membranes of bacteria. Additionally, Prior research has documented

the antimicrobial properties of a few of the compounds that were separated from the exudate of *C. grandiflorus* aerial parts. While MICs ranged from 64 to 88 g/ml, other isolated compounds demonstrated antibacterial efficacy against *Streptococcus mutans*. Because they have biological properties, including antioxidant and antibacterial properties, these secondary metabolites help safeguard plants [42].

Natural antioxidants are helpful in the treatment of many illnesses since they have less harmful side effects and can guard against oxidative deterioration. It has been noted that the plant's extracts and fractions have antioxidant qualities. According to the antioxidant study's findings, *A. fistulosus* is a perfect source of antioxidants because it contains polyphenols. In a number of nations, including Cyprus, Egypt, Libya, Palestine, and Spain, *A. fistulosus* has long been utilized to treat fungal and dermatomycoses diseases [43].

Asphodelus spp extracts have antiseptic and anti-inflammatory properties, antifungal and antitumor activities. Anthraquinones, flavonoids, terpenes, and phenolic acids are the most important metabolites. These metabolites could be responsible for the antiprotozoal activity of this plant. From the results of the present study, different plants of *R. vesicarius* have variable anti-microbial and antioxidant activities. These variations were closely related to the variation in chemical composition within different plants and to the anti-inflammatory activities of compounds isolated from *Eucalyptus globulus* Labill [19].

Conclusions

Herbal medications in traditional medicine often consist of a single herb or a combination of herbs believed to have complementary and/or synergistic effects. The quality of the raw ingredients, which again depend on herbal products, is influenced by factors such as the mineral composition of the soil, geographic location, etc., and determines the quality of the final product. The bioactivities of these herbs are primarily attributed to the high concentration of phenol-based chemicals found in plants. The chemical composition of these plants was determined for the first time using liquid chromatography-electrospray ionization-time-of-flight mass spectrometry analysis (LC-ESI-TOF-MS). 22 chemicals were tentatively identified by LC-MS/MS.

Acknowledgments

The authors are grateful to Dr. Ghalia S. M. Aljeddani, Department of Biology, College of Science, University of Jeddah, Jeddah, Saudi Arabia, for the plant identification according to the Saudi Arabian flora and authentication

Conflict of Interest

The authors declare no conflict of interest.

References

- COLLENETTE S. Wild flowers of Saudi Arabia; National Commission for Wild life Conservation and Development (NCWCD), King Saud University Presses: Riyadh, Saudi Arabia, 799, 1999.
- TONISI S., OKAIYETO K., HOPPE H., MABINYA L.V., NWODO U.U., OKOH A.I. Chemical constituents, antioxidant and cytotoxicity properties of *Leonotisleonurus* used in the folklore management of neurological disorders in the Eastern Cape, South Africa. 3Biotech. **10**, 141, 2020.
- HUSSEINA S.A.M., EL-MESALLAMY A.M.D., EL-ZAIDYB M.I.M., EL-GARBY M.Y., SOLIMANC A.M. Bioactive Compounds from Leaves and Bolls Extracts of *Gossypium Barbadense* L. And Assessment of Their Antioxidants & Cytotoxic Activities. Egyptian Journal of Chemistry. **66** (13) 1117, 2023.
- QARI S.H., ALREFAEI A.F., FILFILAN W., QUMSANI A. Exploration of the Medicinal Flora of the Aljumu Region in Saudi Arabia. Applied Sciences. **11**, 7620, 2021.
- AL-EISAWI D.M., AL-RUZAYZA S. The Flora of Holy Mecca District, Saudi Arabia. International Journal of Biodiversity and Conservation. **7**, 173, 2015.
- AYOUB K., EMIRA N., SOUMIA B., KAISS A., BOUSLAMA L., MOHD A., ANDREA D., ADEL K., MEJDI, S., MUSHTAQ A.K., INES M. LC-ESI/MS-Phytochemical Profiling with Antioxidant, Antibacterial, Antifungal, Antiviral and in Silico Pharmacological Properties of Algerian *Asphodelus tenuifolius* (Cav.) Organic Extracts. Antioxidants. **10**, 628. 2021.
- ODHAV B., KANDASAMY T., KHUMALO N. Screening of African Traditional Vegetables for Their Alpha-Amylase Inhibitory Effect. Journal of Medical and Plants Research. **4**, 1502, 2010.
- ABDEL KHALIK K., AL-GOHARY. I., AL-SODANY Y. Floristic Composition and Vegetation: Environmental Relationships of Wadi Fatimah, Mecca, Saudi Arabia. Arid Land Research and Management. **31**, 316, 2017.
- SHARMA T. Phytochemical screening of medicinal plants and study of the effect of phytoconstituents in seed germination. Tribhuvan University Journal. **35** (2), 1. 2020.
- YU X., YANG T., QI Q., DU Y., SHI J. LIU X., LIU Y., ZHANG H., ZHANG Z., YAN N. Comparison of the contents of phenolic compounds including flavonoids and antioxidant activity of rice (*Oryza sativa*) and Chinese wild rice (*Zizania latifolia*). Food Chemistry. **344**, 128600, 2021.
- DIMKI'C I., PETROVI'C M., GAVRILOVI'C M., GAŠI'C U., RISTIVOJEVI'C P., STANKOVI'C S., JANA'CKOVI'C P. New perspectives of purple star thistle (*Centaurea calcitrapa*) leaf extracts: Phytochemical analysis, cytotoxicity and antimicrobial activity. AMB Exprenet. **10**, 183, 2020.
- MOHIUDDIN A.K. Effect of Environment on Secondary Metabolism of Medicinal Plants. Journal of Environmental and Soil Sciences. **2** (1), 145, 2019.
- NEUPANE P., LAMICHHANE J. Estimation of total phenolic content, total flavonoid content and antioxidant capacities of five medicinal plants from Nepal. Vegetos. **33**, 360, 2020.
- TALHA A.C., MUHAMMAD S., KANWAL R., TARIQ M., MUHAMMAD U.G., KASHIF M.K., IFTIKHAR A.F., MUHAMMAD S.H.A., ALAMGEER I., ARIF M., TAHIR A.C. Phytochemical profiling, antioxidant and antiproliferation potential of *Euphorbia milii* var.: Experimental analysis and in-silico validation. Saudi Journal of Biological Sciences. **27** (11),3025, 2020.
- SAHAR A.M.H., AMANI M.D E., ABDELMOHSEN M.S., MOHAMED E., EL GERBY M. Bioactive compounds from Mango Peels (*Mangifera Indica* Tommy Atkins) and demonstration of its cytotoxicity and Ccl4 induced hepatotoxicity in rats. Egyptian Journal of Chemistry. **64** (9), 4747, 2021.
- CHOUDHARI A.S., MANDAVE P.C., DESHPANDE M., RANJEKAR P., PRAKASH O. Phytochemicals in cancer treatment: from preclinical studies to clinical practice. Frontiers in Pharmacology. **10**, 1614, 2020.
- ALSAEDI S., ALJEDDANI G. Phytochemical analysis and bioactivity screening of primary and secondary metabolic products of medicinal plants in the valleys of

- Medina Region Saudi Arabia. *Advances in Biological Chemistry*. **12**, 92, **2022**.
18. ALJEDDANI G.S.M., ALMOSHADAK A.S. Pomegranate peel ethanolic extract: A promising natural antioxidant, antimicrobial agent, and novel approach to mitigate rancidity in used edible oil. *Open Agriculture*. **9**, 20220350, **2024**.
 19. BASUDAN N.S., QAHTAN N.K. LC/MS/MS analysis and In Vitro- Phytochemical profiles of two wild plants from Saudi Arabia. *Egyptian Journal of Veterinary Sciences*. **55** (7), 1939, **2024**.
 20. MEKNI M., AZEZ R., TEKAYA M., MECHRI B., HAMMAMI M. Phenolic non-phenolic compounds and antioxidant activity of pomegranate flower, leaf and bark extracts of four Tunisian cultivars. *Journal of Medical Plants Research*. **7**, 1100, **2013**.
 21. BASUDAN N.S., DANIAL E.N. In-vitro comparative phytochemical screening, antimicrobial, antioxidant and LCMS/MS analysis of *Ocimum basilicum* and *Origanum vulgare* extracts in Saudi Arabia. *Egyptian Journal of Veterinary Sciences*. **55** (3), 681, **2024**.
 22. SHAKER K.H., ZOHAIIR M.M., HASSAN A.Z., SWEELAM H.M., ASHOUR W.E. LC–MS/MS and GC–MS based phytochemical perspectives and antimicrobial effects of endophytic fungus *Chaetomium ovatoascomatis* isolated from *Euphorbia milii*. *Archives of Microbiology*. **204**, 661, **2022**.
 23. TIWARI P., KUMAR B., KAUR M., KAUR G. and KAUR H. Phytochemical screening and extraction: A Review. *Internationale Pharmaceutica Scientia*. **1** (1), 98, **2011**.
 24. MUHAMMAD K.G., MUHAMMAD A.G., SAJID N.H., MAJID M., MUKHAYAR A., WAJID S. Ethnopharmacological, phytochemical and pharmacognostic potential of Genus *Heliotropium* L. *Turki Journal of Pharmaceutical Sciences*. **13** (2), 259, **2016**.
 25. BARBOUCHI A.M., BENZIDIA B., ELAMRANI K., SABIRI M., EL IDRISSE M. Phytochemical screening, quantitative analysis and antioxidant properties of crude extracts from stems, leaves, and flowers of three *Ruta species*. *Kuwait Journal of Science*. **51**, 100287, **2024**.
 26. HARBORNE J.B. *Phytochemical methods*. Chapman and Hall Ltd., London, pp.100, **1998**.
 27. SINGH V., KUMAR R. Study of Phytochemical analysis and antioxidant activity of *Allium sativum* of Bundelkhand Region. *International Journal of Life Sciences Scientific Research*. **3** (6), 1451, **2017**.
 28. KHAN S., ALGHAFARI Y., KHA S. Temperature and precipitation fluctuation of Madinah-Al-Munawara, Kingdom of Saudi Arabia (1959-2011). *Atmospheric and Climate Sciences*. **6** (3), **2016**.
 29. AL MASOUDI L.M., ALQURASHI A.S., ABU ZAID A., HAMDI H. Characterization and biological studies of synthesized Titanium dioxide nanoparticles from Leaf Extract of *Juniperus phoenicea* (L.) Growing in Taif Region, Saudi Arabia. *Processes*. **11**, 272 **2023**.
 30. SHAIKH J.R., PATIL M.K. Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*. **8** (2), 603, **2020**.
 31. TENIENTE S.L., FLORES-GALLEGOS A.C., ESPARZA-GONZÁLEZ S.C., CAMPOS-MÚZQUIZ L.G., NERY-FLORES S.D., RODRÍGUEZ-H.R. Anticancer effect of Pomegranate Peel polyphenols against cervical cancer. *Antioxidants*. **12** (1), 127, **2023**.
 32. GANCEDO N.C., ISOLANI R., DE OLIVEIRA N.C., NAKAMURA C.V., DE MEDEIROS ARAÚJO D.C., SANCHES A.C.C., TONIN F.S., FERNANDEZ-LLIMOS F., CHIERRITO D., DE MELLO J.C.P. Chemical constituents, anticancer and anti-proliferative potential of Limonium Species: A Systematic Review. *Pharmaceutics*. **16**, 293, **2023**.
 33. SKROVANKOVA S., SUMCZYNSKI D., MLCEK J., JURIKOVA T., SOCHOR J. Bioactive compounds and antioxidant activity in different types of berries. *International Journal Molecular Sciences*. **16** (10) 24673, **2015**.
 34. MWAMATOPE B., TEMBO D., CHIKOWE I., KAMPIRA E. and NYIRENDA C. Total phenolic contents and antioxidant activity of *Senna singueana*, *Melia azedarach*, *Moringa oleifera* and *Lannea discolor* herbal plants. *Scientific African*. **9**, 00481, **2020**.
 35. RADWAN D.E.M., EL-SHABASY A.E. Comparative analysis of five *Heliotropium* species in phenotypic correlations, biochemical constituents and antioxidant properties. *The Egyptian Society for Environmental Sciences*. **21** (1), 1, **2020**.
 36. KUMARAN A., KARUNAKARAN R.J. In vitro antioxidant activities of methanol extracts of five *Phyllanthus* species from India. *LWT - Food Science and Technology*. **40** (2), 344, **2007**.
 37. ABD-ELGAWAD A.M., ELSHAMY A.I., AL-ROWAILY S.L., EL-AMIER Y.A. Habitat affects the chemical profile, allelopathy, and antioxidant properties of essential oils and phenolic enriched extracts of the invasive plant *Heliotropium curassavicum*. *Plants*. **8** (11), 482, **2019**.
 38. EL-HAWARY S., SOKKAR N.M., ALI Z.Y., YEHIA M.M. A profile of bioactive compounds of *Rumex vesicarius* L. *Journal of Food Science*. **76** (8), 1195, **2012**.
 39. EL-ANSSARY A.A., ABDEL RAOOF G.F., SALEH D.O., EL-MASRY H.M. Bioactivities, physicochemical parameters and GC/MS profiling of the fixed oil of *Cucumis melo* L seeds: A focus on anti-inflammatory, immunomodulatory, and antimicrobial activities. *Journal of Herbed Pharmacol*. **10** (4), 476, **2021**.
 40. EL-SHABRAWY M.O., MARZOUK M.M., KAWASHTY S.A., HOSNI H.A., EL GARF I.A., SALEH N.A.M. Flavonoid constituents of *Dipcadi erythraeum* Webb. & Berthel. *Asian Pacific Journal of Tropical Disease*. **6** (5), 404, **2016**.
 41. BREZANI V., LELAKOVA V., HASSAN S., BERCHOVA-BIMOVA K., NOVY P., KLOUCEK P., MARSIK P., DALL'ACQUA S., HOSEK J., SMEJKAL K. Anti-infectivity against Herpes simplex virus and selected microbes. *Viruses. Pharmacology and Pharmacy*. **10** (7), 360, **2018**.
 42. SHEHABELDINE A.M., ASHOUR R.M., OKBA M.M., SABER F.R. *Callistemon citrinus* bioactive metabolites as new inhibitors of methicillin-resistant *Staphylococcus aureus* biofilm formation. *Journal of Ethnopharmacology*. **254**, 112669, **2020**.
 43. FARID M.M., SALEM M.A., ABD EL-LATIF R.R., ELKHATEEB A., ABDEL-HAMEED E.S., MARZOUK M.M., HUSSEIN S.R. Chemical analysis and cytotoxic evaluation of *Asphodelus aestivus* Brot. flowers. *Egyptian Journal of Chemistry*. **64** (9), 51, **2021**.