Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

LC/MS/MS Analysis and In-Vitro Phytochemical Profiles of Two Wild Plants From Saudi Arabia

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Abstract

Medicinal plants (MP) have been demonstrated to be a plentiful spring of biological vigorous compounds for the improvement of novel indication compounds for drugs. Screening of *Artemisia judaica (A.judaica*) and *Lavandula dentate* (*L.dentate*), plant extracts approve the occurrence of several phytochemicals alike carbohydrates, cardiac glycosides, coumarins, flavonoids, tannins, terpenoid, steroids and phenols in the selected plants. While other phytochemicals such as anthraquinone, alkaloids, saponins, proteins, and phytosterols were detected and varied based on the plant constituents. The antioxidant, antibacterial and antifungal actions of the plant extract were recognized. The antioxidant activity was assessed by DPPH. The antimicrobial action was bioassayed by the agar well diffusion technique. The results approved the character of these extracts as talented, strong antioxidants and moderate antimicrobial representatives. The impartial of this work was to conclude the phytochemical broadcast, investigation by LC-MS/MS, the antioxidant, antimicrobial activities of *A. judaica* and *L.denta*, ethanolic extract acquired from AlBaha in the Kingdom of Saudi Arabia.

Keywords: *Artemisia judaica* and *Lavandula dentate*, phytochemical broadcast and analysis, antioxidant, antimicrobial actions.

Introduction

The term medicinal plants (MP) include various types of plants utilized in herbalism and approximately of these plants have therapeutic activity. The environmental factors, and habitat-related effects, associated with high salinity, desert climate, and water and nutrient scarcity have emphasized the importance of halophytic plants in the fields of drug discovery, and alternative medicines MP is the "support" of traditional medicine (TM), which means more than 3.3 billion public in the less developed nations use MP on a steady source [1]. About 80% of people in the world use TM for their health. The normal utilization of TM in essential medical care ought to be founded on the rules for the evaluation of homegrown prescriptions as evolved by the World Health Organization [2].

The natural products that got from MP have shown to be a bountiful wellspring of biologically active compounds, a significant number of which have been the reason for the improvement of new lead synthetic substances for drugs [3]. The historical backdrop of TM is basically as old as human civilization. The earliest records affirm that homegrown meds have been utilized and reported in Roman, Greek, Egyptian, Chinese, and Indian restorative frameworks for around 5000 years. MP is used on a large scale in many countries (developing or primitive) because of its biological effects on organisms. Industrial, there are many uses of MP, either it was TM, herb therapy, or health food. The active role of MP lies in the existence of some active compounds such as: (phenol, flavone, coumarins, alkaloids… etc.) that are considered sources of many drugs in pharmaceuticals. Starting from the beginning of history, plant plays had a significant influence on the treatment of human infirmities. By experimentation, the old populace was easing their enduring by involving spices in an extremely crude manner [4]. It is misleading to describe Saudi Arabia "barren desert" when it is there many regions clothed with trees, herbs, and flowers as those in the south and some of the west area. Most of these plants have effective effects from a medical point of view, but in my research, I highlight aromatic plants that are having biological importance.

An example of these plants, *A. judaica* is abundant in the south of Saudi Arabia. Saudi Arabia

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(Received 09/01/2024, accepted 16/03/2024)
DOI: 10.21608/EJVS.2024.260484.1763

has a high-salinity ecosystem, which affects plants' growth, and is a significant challenge behind the slowed development of agriculture in the area especially in Jizan. There are Lavandula widely found stretching from north to the end of the Sarwat mountain range in the south of the Kingdom of Saudi Arabia, and from the famous plants in those areas.

Experimental

Collection of the herb leaves

The environmental factors, and habitat-related effects, associated with high salinity, desert climate, and water and nutrient scarcity have emphasized the importance of halophytic plants in the fields of drug discovery, and alternative medicines Two herbs (*A. judaica* and *L. dentate*) were collected from Al-Baha City Saudi Arabia, the leaves of these herbs were taken and chopped softy.

Herbal Extraction

The two plant leaves were collected and finely grinded after they are dried in room temperature. Then taken the weights of all the leaves. Plants were macerated two times with ethanol (75% concentration) for 48 hr and filtered (**Table 1**).

Phytochemical screening

The extract was prepared by dissolving 0.1 gm in 5ml of distilled water and filtrating.

Carbohydrates

Fehling's test, Benedict's test and and Molisch'stest, Steroids (Liebermann-Burkhardt test), Cardiac glycosides (Keller-Killiani test), Terpenoids (Salkowski's test), Anthraquinone (Bontrager's test), Coumarins, Flavonoids and Tannins. Method used according to Khayyat [5].

Proteins

Biuret test and Ninhydrin test, **Xanthoproteic test, Alkaloids Phytosterols, Saponins, Phenols.** Method used according to Silva and Abeysundara [6].

Antimicrobial evaluation

The antibacterial activity of the plant extract (A. Judaica and L. Dentate fruit 5g crude extracts in 100 ml of d H_2O) was investigated by the well diffusion method [7]. The assay was carried out with four bacterial species: *Escherichia coli* ATCC 25922 (American Type Culture Collection),

In-Vitro Determination of Antioxidant Activity

Free-radical scavenging activity: DPPH assay

The capacity of the prepared plant extracts to scavenge the 'stable' free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was monitored according to the method described by Mekni *et al*. [8].

Determination of total phenolic content

The total phenolic content of the plant extract was determined using the Folin-Ciocalteu method described by Mutahar *et al.* [9].

LC/MS/MS

Mass Spectrometry (MRM) analysis of the selected polyphenols, positive and negative ionization modes were applied in the same run with the following parameters: curtain gas: 25 psi; Ion Spray voltage: 4500 and-4500 for positive and negative modes, respectively; source temperature: 400°C; ion source gas 1 & 2 were 55 psi with a declustering potential: 50; collision energy: 25; collision energy spread:

Results and Discussion

Phytochemical Screening

Phytochemical components of the plants premeditated were examined for the subsequent metabolites: anthraquinone, alkaloid, carbohydrate, cardiac glycosides, coumarins, flavonoid, tannin, saponins, terpenoid, steroid, protein ،phytosterols and phenol. Trace elements deficiency may result from either a decrease in specific element's intake, or an increase in the levels of these elements due to any biochemical, physiological, or environmental causes, whereby both may lead to impairment, regression, and higher activity of the biochemical pathways. The results of phytochemical screening were presented in following (Table 2).

Qualitative broadcast consuming ethanolic extract showed the occurrence of greatest the phytochemical ingredients and the absence, other of it in plants. As it is seen from the table (2), the constituents were revealed to present in varying proportions in plants. Note that, the resulting colors may increase (>+), remain Light (+), or is absent (-) according to the results observed during the experiment.

We also note that phytochemical that is the most abundant in all plants were cardiac glycosides, as the very dark brown ring, appeared on all plants (++ or+++) ،which can be considered the plants as having an active effect as antitumor activity and inhibitory activity.

The preliminary phytochemical screening of ethanolic extracts (96%) of the selected plants tabulated in Table 2 showed that *A. judaica* contains carbohydrates, terpenoids, and Cardiac glycosides in abundant quantity, while the tannins, flavonoids, terpenoids resins, and saponins, alkaloids with a moderate quantity, but it is free from anthraquinone and phytosterols. Some bioactive MP polysaccharides are relatively nontoxic and do not cause significant side effects, have attracted a great deal of attention in pharmacology and the

biochemistry field. The high contents of trace elements in these halophytic plants are also considered responsible for supplementing their antioxidants and supporting several other biological actions. So it have potential biological activities such as antitumor, anticoagulant, antioxidant, antidiabetic, and immunomodulatory activities [10].

A. Judaica was phytochemically tested for secondary plant metabolites, tannins, alkaloids, flavonoids, saponins, and total phenolic compounds [11]. The presence of these phytochemicals in the herb has been proven varying proportions.

The Preliminary phytochemical screening of ethanolic extracts (96%) of the plants tabulated in Table 2 showed that *L. dentate* contains Cardiac glycosides, terpenoids, and phenols in abundant quantity, while carbohydrates, Coumarins, flavonoids, tannins, steroids, proteins, and phytosterols with a moderate quantity, but it is free from anthraquinone, alkaloids, and saponins. The phenolic compounds are the potential candidate bioactive agents in pharmaceutical and medical sectors as an antioxidant effect, antibacterial effect, anti-cancer effect, cardioprotective effects, immune system, promoting and anti-inflammatory effects, and skin protective effect of UV radiation [12].

There are various reports about the combination of components and most of them in different parts of the world have been shown to be predominantly phenolic compounds. Coumarins, tannins, flavonoids, phenols, and fatty acids were found in phytochemical screening [13].

The results in Table 2 indicated that the selected plant extract contains terpenoids and cardiac glycosides. Most of the terpenoids may be applied worldwide, especially for the treatment of various bacterial infectious diseases and as anticancer drugs [14]. Some cardiac glycosides are used as an antitumor activity and an inhibitory activity against rhinovirus [15]. The difference in the proportion of the presence of phytochemicals and the absence of plants depends on the environmental factors, and habitat-related effects, associated with high salinity, desert climate, and water and nutrient scarcity have emphasized the importance of halophytic So, these constituents may be absent and appear on the same plant-based for these reasons.

Antimicrobial activity of *Artemisia judaica and Lavandula*

The antimicrobial properties of *A. judaica* and *L. dentate* ethanolic extracts on the growth of several Gram +, G- bacteria, and fungi using the agar diffusion method are shown in Table 3 and Fig (1). These results agreed with the Nasr, [16] denoted that the *A. judaica* plant extract exhibited weak antimicrobial activity against G+ and G– bacteria. About the antifungal activity, the extract inhibits the growth of *C. albicans* with inhibitory activity of 14 mm and 12 mm against *A.* niger. In A. Judaica fruit extracts, the phytochemical analysis revealed the presence of phenolics, flavonoids, steroids, triterpenoids, proteins, alkaloids, and saponins (Table 2), which could contribute to their antibacterial action. Antibacterial activity may be attributed to phenolics, flavonoids, and steroids. Many studies on phenolics and flavonoids have been published, Saponins exhibit a biological role and medicinal properties such as antibacterial, antifungal and steroidal glycoside [13] influenced antibacterial action. Alkaloids are the most important active compounds in herbs. They display antimicrobial properties, and antifungal proteins, which inhibit the growth of fungi [17].

As well as, the results indicated that the *L. dentate* showed moderate inhibitory activity against all the tested pathogens with IZ ranging from 12 to 16 mm. (Table 3).

Our results were in accordance with Bogdan et al [18] discovered that Lavandula derived from the three analyzed varieties of Lavandula angustifolia has substantial bactericidal and antifungal activities against the tested pathogens (*S. aureus and E. coli*) as well as antifungal properties against Candida albicans. Meanwhile, the antimicrobial activity of *A. judaica* and *L. dentate* species may be evaluated based on extraction efficiency as well as their active compound location. In contrast, our results showed that the ethanol extract of *A. judaica* and *L. dentate* inhibited all the test organisms with a different degree of inhibitory action. This difference may be attributed to location or seasonal variations.

Investigating various methods for developing new antibacterial products for the treatment of drug-resistant bacterial infections have recently attracted attention due to the growth of antibiotic-resistant bacteria and the lack of new antibiotics available on the market Masu. Because of the antibacterial qualities of plant extracts, they may be able to aid in the reduction of bacterial resistance [19].

Antioxidant activity of *A. judaica and L. dentate*

Free radical scavenging DPPH assay

A quantitative analysis using radical scavenging DPPH assay was followed by the standard protocol of Mekni [8]. The result obtained from the antioxidant activity of the A. Judaica and *L. dentate* extracts showed in Table 4. and Figure 1. It shows that the antioxidant activity of both plant extracts was more than 50%. They consider having strong antioxidant activity.

In the case of *A. judaica*

Antioxidants are promising therapeutic agents in wound healing [20]. The majority of wound-healing plants have a noteworthy antioxidant efficacy, which has been investigated using various in vitro and in vivo assays [21]. The results obtained in Table 4 showed the antioxidant activity of the A. Judaica determined by radical scavenging DPPH assay

indicated that the inhibition is 90 %. These results were in accordance with Massry, *et al.* [15] those who have shown that the volatile oils of *A. Judaica* L have significant antioxidant activity and show their effectiveness against diseases caused by overproduction of free radicals. In vitro, similar studies reported the plant extract's potent antioxidant and anticancer activities [16]. As well, the essential oils acquired from numerous plants of the species Artemisia exhibited potent free radical scavenging, and reduction [22].

In the case of *L. dentate*

The antioxidant activity of the samples determined by radical scavenging DPPH assay indicated that the inhibition is 89 %. The obtained outcome was more than that obtained by Bogdan et al [18] who detonated that the antioxidant activity of the *L. dentate* determined by the DPPH assay indicated that the inhibition is varying from 32.37 to 69.83% for DPPH, contingent on the diversity and year of the gathering of the plant. Antioxidant activity depends, on the one hand, on the quality of the original plant, its geographical origin, and climatic conditions, genetic and growth conditions such as harvest date, storage, and processing, and on the other hand, the extraction method and its specified factors [23].

The lavender dentate extract obtained by the extraction shows the possibility of incorporation into pharmaceutical products and antioxidant/ antimicrobial additives are desired and to find possible alternatives to synthetic preservatives. The antioxidant activity of the extract is due to the phenolic compounds present. Differences in antioxidant activity can be attributed not only to the interaction of phenolic compound extracts, but also to structural diversity [24].

Total phenolic content

The concentration of phenolic in the two plant extracts was presented in Table 4 and Figure 2 was dependent on the solvent and the experimental conditions. The total phenolic content was expressed as Galic acid equivalents (GAE) in milligrams per gram of extract . The amount of phenolic compounds in the *A. judaica* plant ethanol extract was (8.96 mg GAE/1g of extract). These results were lower than the value obtained by Allam et al. [25] who indicated that the TPC varied between 174.82 ± 0.38 and 248.93 ± 0.34 mg GAE g−1 DE in the extracts. The presence of phenolics and flavonoids in high amounts in this extract may be responsible for the free-radical scavenging activity.

The amount of phenolic compounds in the *L. dentate* plant ethanol extract (27.32mg GAE/1g of extract) was the highest. The results were most nearly to that obtained by Bajalan *et al.* [26] who reported that total phenolic contents varied from 31.45 to 105.39 (mg GAE/100 g dry wt).

Previous research has investigated the relationship between total phenolic content and antioxidant capabilities of a variety of plants [27]. Some studies found good positive linear relationships; others found poor linear correlations or couldn't explain the relationship between total antioxidant activity and Phenolic concentration, as Mata *et al.* [28] reported, and as we found in our research.

LC-MS/MS Analyses

The *A. judaica* and *L. dentate* extracts were established by LC-MS/MS. Their composition in µg/ml was shown in Table 5, 6, and Figures 3-4 .The most abundant component in *A. judaica* (µg/ml) were Naringenin 520; Chlorogenic acid, 151; Caffeic acid, 27; 3, 4 dihydroxybenzoic acid, 13.50; Luteolin 11.44, Apigenin 6.73 and Rutin 5.57 (Table 5 and Fig.3)

These findings matched those of Moharram et al. [29], who discovered flavonoids such as apigenin, aglycones and glycosides, quercetin, and luteolin. Other common classes, such as triterpenes, phenolics, bitter principles, and sesquiterpene lactones, such as judaicin, have also been discovered in the plant.

The results indicated that the most abundant components (µg/ml) in *L. dentate* extract were Caffeic acid, 181; Chlorogenic acid, 27.70; 3.4-Dihydroxybenzoic acid, 33.73, Naringenin, 31.68; Luteolin, 27.80; Coumaric acid, 19.69 and Ferulic acid 6.65. (Table 6 and Fig.4). The results obtained were compared with the results of Da Porto et al. [30] 60 compounds were identified from lavender essential oil. This was more than the 21 components identified in this study. As previously stated, the chemical composition of essential oils reported in different research is likely due to variances in extraction, operating parameters as well as chemical components of lavender, which are impacted by variety, growth conditions, and harvesting time, hence the phenological stage of the plant, environmental differences within species [31].

Conclusion

In the present study, phytochemical screening revealed the presence of carbohydrates, cardiac glycosides, phenolic acids, coumarins, saponins, flavonoids, tannins, terpenoids, steroids, anthraquinone and alkaloids, and phenols in the two selected herbs and their extract showed moderate a scavenging radical activity. The ethanol plant extracts exhibited noticeable activity against gram-positive and gram-negative bacteria and fungi. LC-MS/MS analyses of these two plants were performed and identified 21 bioactive compounds. The extracts of *A. judaica* and *L. dentate* have the potential to be

alternatives In vivo study, applied on experimental animal can confirm this investigation to medicinal and food protection systems in later investigation.

Acknowledgement: Not applicable

Conflict of interest: All Authors declare that there is no conflict of interest.

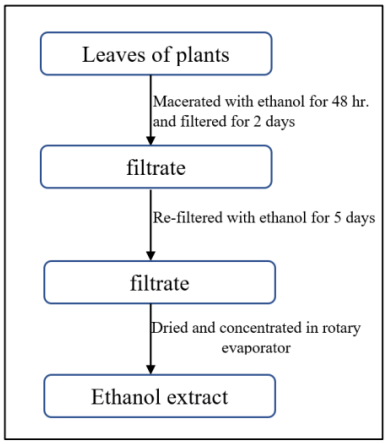
Funding statement: No funding was received for conducting this study

Author`s contributions: Conceptualization, study design, sample collection, and Ultrasonography: Nour Basudan. Data analyses, Manuscript drafting, and Manuscript finalization: Nedaa Qahtan.

TABLE 1. Plants weight and the amount of solvent involved in the extraction process

No	Plants	Weight (gm)	Solvent (EHOH)	
First time (ml)	**second time (ml)**		First time (ml)	Second time (ml)
1	*A. judaica*	100	360	450
2	*L. dentate*	200	600	500

The standard extraction scheme used during the study is shown below:



Skim 1. Filtration process...

TABLE 2. Phytochemical screening of ethanol extract of *A. judaica* and *L. dentate*

Plants Phytoconstituents		
Compounds	***A. judaica***	***Lavandula***
Anthraquinone	-	-
Alkaloid	+	-
Carbohydrate	++	+
Cardiac glycosides	++	++
Coumarins	+	+
Flavonoid	+	+
Tannins	+	+
Saponins	+	-
Terpenoids	++	++
Steroids	+	+
Proteins	+	+
Phytosterols	-	+
Phenols	+	+++

TABLE 3. Phytochemical screening of ethanol extract of *A. 1944udaica* and *L. dentate*

Plant	Gram Positive bacteria		Gram Negative bacteria		Fungi	
	B. Subtilis	*S. aureus*	*E. coli*	*P. arogenos*	*C. albicans*	*A niger*
A. judaica	16	00	16	14	14	12
L. dentate	20	16	14	16	14	16

Table 4. Antioxidant Activity DPPH% and Total phenolic compounds of ethanol extract of *A. judaica* and *L. dentate*

Plant	Antioxidant Activity DPPH%	Total Phenolic (mgGA/1 gdwt)
A. judaica	90.14	8.96
L. dentate	89.04	27.32
Galic acid	66.59	4.44

TABLE 5. LC/MS/MS of *A. judaica* ethanolic extract

Name	Precursor m/z		Retention Time (Rt)		Conc. (µg/1gm plant extract)	
	A. judaica	*L. dentate*	*A. judaica*	*L. dentate*	*A. judaica*	*L. dentate*
Chlorogenic acid	355.1/163	355.1/163	7.34	7.34	151.16	27.70
Daidzein	255.1/199	255.1/199	12.93	12.93	ND	ND
Gallic acid	168.9/124.9	168.9/124.9	3.85	3.85	0.72	2.43
Caffeic acid	178/135	178/135	8.04	8.04	27.92	181.01
Rutin	609/299.9	609/299.9	9.72	9.72	5.57	0.06
Coumaric acid	162.9/119	162.9/119	9.53	9.53	3.50	19.69
Vanillin	151/136	151/136	9.57	9.57	4.07	2.91
Naringenin	271/119	271/119	15.05	15.05	519.96	31.68
Querectin	301/151	301/151	13.59	13.59	1.97	0.09
Ellagic acid	301/145	301/145	9.92	9.92	ND	0.58
3.4-Dihydroxybenzoic acid	152.9/109	152.9/109	5.72	5.72	13.49	33.73
Hesperetin	301/136	301/136	15.64	15.64	ND	3.60
Myricetin	317/137	317/137	11.72	11.72	ND	ND
Cinnamic acid	146.9/102.6	146.9/102.6	14.20	14.20	ND	ND
Methyl gallate	183/124	183/124	7.45	7.45	ND	ND
Kaempferol	284.7/93	284.7/93	15.36	15.36	0.20	0.19
Ferulic acid	192.8/133.9	192.8/133.9	10.25	10.25	1.85	6.65
Syringic acid	196.8/181.9	196.8/181.9	8.41	8.41	1.40	16.29
Apigenin	269/151	269/151	15.05	15.05	6.73	2.99
Catechin	288.8/244.9	288.8/244.9	7.34	7.34	ND	ND
Luteolin	284.7/132.9	284.7/132.9	13.52	13.52	11.44	27.80

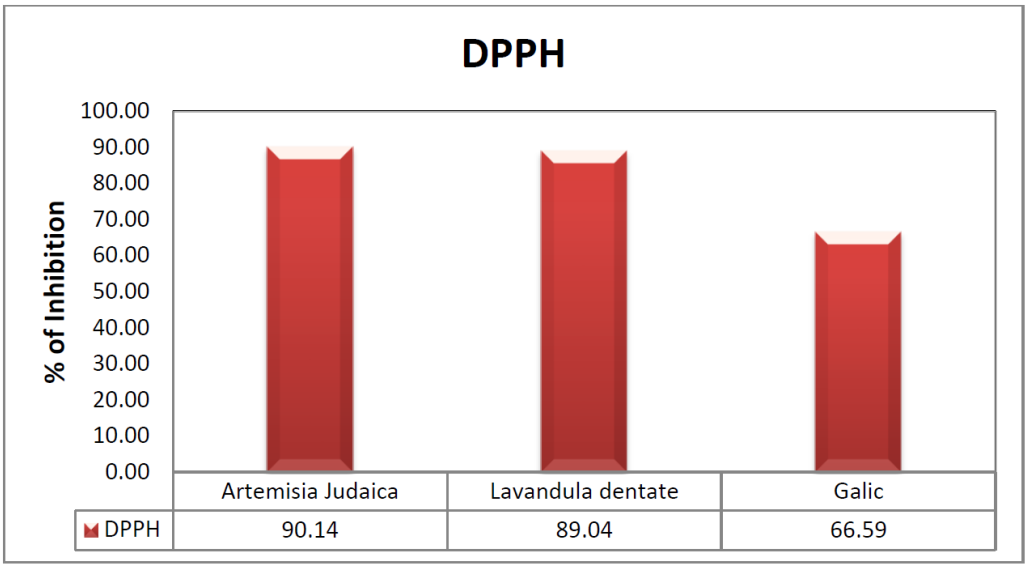


Fig. 1. Antioxidant Activity DPPH% of ethanol extract *of A.judaica* and *L. dentate*

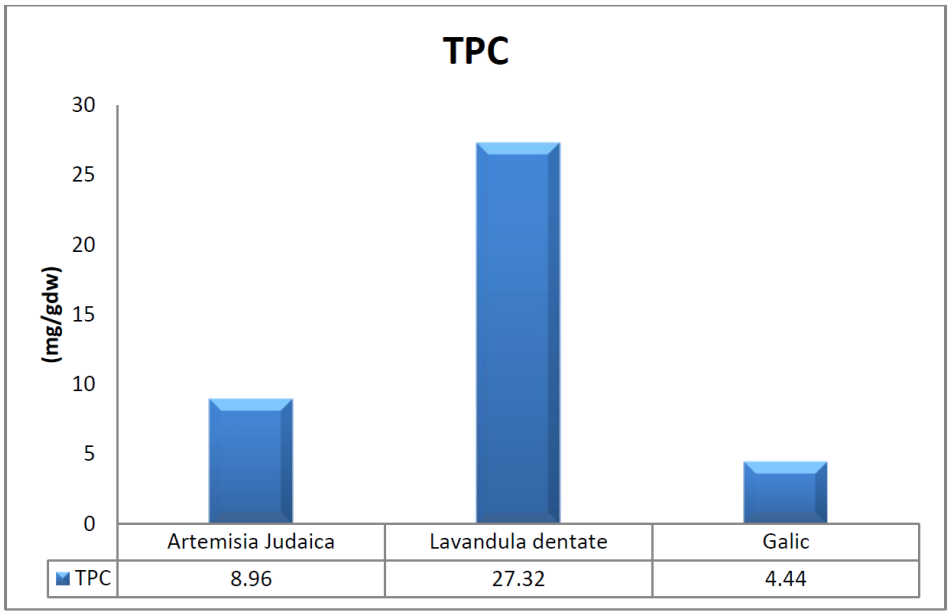


Fig. 2 Total phenolic compounds of ethanol extract for *A. judaica* and *L. dentate*

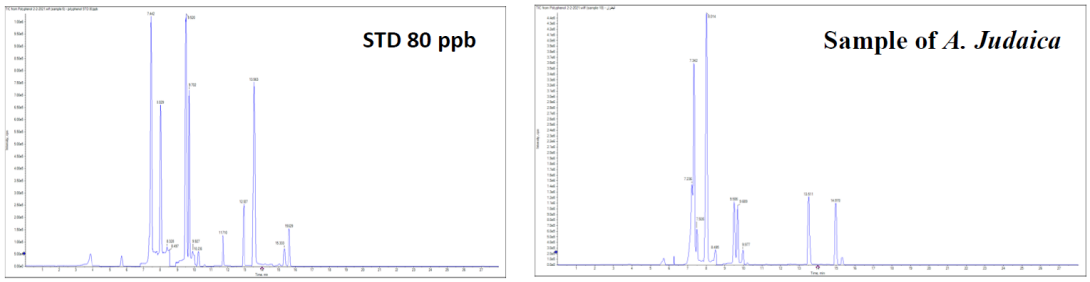


Fig. 3. LC/MS/MS of *A. judaica* ethanolic extract

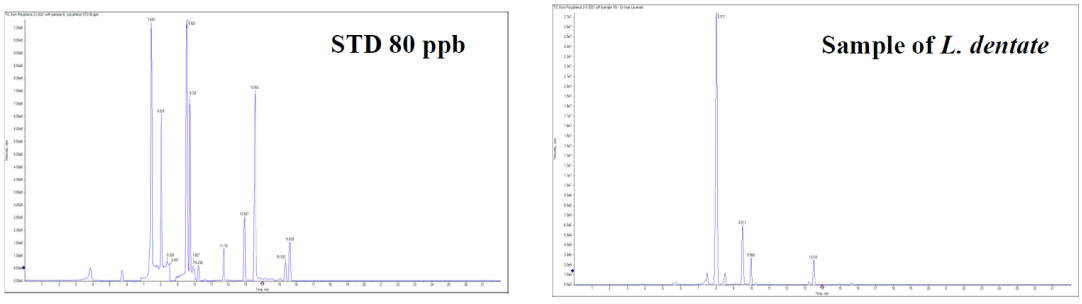


Fig. 4. LC/MS/MS of *L. dentate* ethanolic extract

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تحليل LC/MS/MS والمحتوي الكيميائي لنباتين بريين من المملكة العربية السعودية

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لقد ثبت أن النباتات الطبية (MP) هي مصدر وفير للمركبات البيولوجية القوية لتحسين مركبات الأدوية الجديدة. أظهرت نتائج فحص نبات Artemisia judaica (A.judaica وL.dentate (Lavandula dentate) وجود العديد من المواد الكيميائية النباتية مثل الكربوهيدرات، الجليكوسيدات القلبية، الكومارين، الفلافونويدات، العفص، التيربينويد، الستيرويدات والفينولات في النباتات المختارة. بينما تم الكشف عن مواد كيميائية نباتية أخرى مثل الأنثراكينون والقلويدات والصابونين والبروتينات والفيتوستيرول وتنوعت بناءً على مكونات النبات. تم التعرف على التأثيرات المضادة للأكسدة والمضادة للبكتيريا والفطريات للمستخلص النباتي. تم تقييم نشاط مضادات الأكسدة بواسطة DPPH. تم إجراء اختبار حيوي للعمل المضاد للميكروبات بواسطة تقنية نشر بئر الأجار. أثبتت النتائج طبيعة هذه المستخلصات كمضادات أكسدة ظاهرة وقوية ومضادات ميكروبات متوسطة. كان الهدف من هذا العمل هو اختتام التركيب الكيميائي النباتي، والتحقيق بواسطة LC-MS/MS، والأنشطة المضادة للأكسدة والمضادة للميكروبات لـ A. judaica وL.denta، والمستخلص الإيثانولي الذي تم الحصول عليه من الباحة في المملكة العربية السعودية

الكلمات الدالة: الشيح ، اللافاندولا ، والتحليل الكيميائي النباتي، مضادات الأكسدة، المضادة للميكروبات.