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Phytochemical screening, total phenolics, antioxidant activity and minerals composition of *Helichrysum stoechas* grown in Libya

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Abstract

Helichrysum stoechas is a popular plant in traditional Libyan medicine used to treat various ailments. Herein, phytochemical studies were carried out on aqueous and ethanol extracts of *Helichrysum stoechas* leaves and stems. The screening confirmed the presence of flavonoids, glycosides, anthraquinones, saponins, tannins and alkaloids. The total content of phenolic compounds and antioxidant activity were measured by the Folin-Ciocalteu method and the Prussian blue method, respectively. Significant values of total phenolic compounds and antioxidant capacity were obtained for the leaves and stems of *Helichrysum stoechas*. The antimicrobial activity against 5 microorganisms was evaluated by the agar diffusion method. The ethanolic leaves extract was active against *Escherichia coli*, and the ethanolic stems extract was active against *Candida spp.* To date, no research has been done on the mineral composition of *Helichrysum stoechas.* The minerals in the plant were analyzed and the data showed that the plant contained high levels of sodium, potassium and calcium and lower levels of iron, copper and nickel.

Key words: *Helicherysum stoechas*; Al- Himadah region; Mineral analysis; Antimicrobial activity.

1. Introduction

Medicinal plants commonly used in the treatment and prevention of diseases (Schulz et al., 2001). Globally, more than 70,000 plant species considered as medicinal plants or at least used in traditional medicine (Farnsworth & Soejarto, 1991; Schippmann et al., 2006). The medicinal value of plants lies in certain phytochemicals and minerals that produce several physiological effects on the humans (Petrovska 2012; Afuape et. al., 2022). The total plant species in Libya about 2103 species distributed in 856 genera and 155 families (Feng et al., 2013; Auzi, 1999). The most dominant and sizable families in Libya are Asteraceae, Fabaceae, Poaceae, and Brassicaceae. Whereas, the families Chenopodiaceae, Liliaceae and Lamiaceae are considered as less dominant (Sherif et al., 2022). According to literature, 450 plant species used for medicinal purposes and they are spread all over Libya, especially in Al-Jabal Al-Akhdar (El-Mokasabi, 2014) due to its unique geography and climate (Al-Idrissi et al., 1996; Al-Sodany et al., 2003).

The genus *Helichrysum* is a member of the Asteraceae family, which is among the largest in the world and the main family found in Libya. This genus encompasses about 600 species of flowering plants. (Hilliard et al., 1983; Al-Idrissi et al., 1996). A large number of the species are used in folk medicine in several cultures across the word for various biological properties including anti-inflammatory antioxidant, antibacterial, antifungal and antiviral bioactivities (Sala, 2002; Taglialatela-Scafati et al., 2012; Minaiyan et al., 2014; Kutluk et al., 2018). Beside medicinal treatment, the herbal plants are used in cosmetic industry (Reidel et al., 2017) as well as in cooking (Ghirardini et al., 2007). The *genus* is widespread in Libya in two species, *Helichrysum lacteum* and *Helichrysum stoechas (*Alavi, 1983).

Helichrysum stoechas grows widely in Libya and used excessively by natives. Biological studies of various parts of the *Helichrysum stoechas* were done. The studies have shown that this plant has strong antioxidant (Carini, et al., 2001), anti-inflammatory (Recio et al., 1991) and antimicrobial (Rios, et al., 1991; Roussis et al., 2002) activities. The plant has also been proposed as a treatment for urinary stones (Orhan, et al., 2015*)* and hypertension (Valero et al., 2022). Due to these biochemical and physiological effects, the phytochemical compounds of the plant have been identified. The first study to isolate phytochemical compounds from *Helichrysum stoechas,* grown in Libya, was reported in 1993 (El-Dahmy, 1993). The report confirmed the presence of five benzofuran derivatives in the *Helichrysum stoechas*. According to numerus studies, the plant is rich in essential oils, which are concentrated in flowers and leaves (Vernin & Poite, 1998; Tsoukatou, et al., 1999; Ascensao et al., 2001), as well as the plant is an important source of phenolic compounds. Previous studies confirmed the presence of phenolic compounds in samples of *Helichrysum stoechas* from Italy, Turkey, Portugal, Algeria, and France (Barros et al., 2010; Albayrak, et al., 2010 ; Haddouchi et al., 2014; Carini et al., 2001; Lavault & Richomme, 2004)

The phenolic compounds have received considerable attention because of their physiological functions (Carini, et al., 2001; Rios, et al., 1991; Sobhy & El-Feky, 2007). The antioxidant activity of phenolics is mainly due to a number of different mechanisms such as free radical-scavenging (Amarowicz et al., 2004), which cause damage to proteins and RNA, leading to the production of cancer cells (Gupta, 2020).The antimicrobials of plant origin are of interest to the pharmaceutical

industry for their resistance to microbial pathogens and because bacteria and fungi are always trying to find new ways to avoid the effect of antimicrobial drugs, researches are continuing to obtain useful natural antimicrobials (Khameneh, 2019).

Minerals are essential components for health. They are needed in very small amounts for most biochemical processes. 10 essential elements that our body must contain appropriate amounts of them, which are Na, K, Mg, Ca, Fe, Mn, Co, Cu, Zn, and Mo. (Zoroddu et al., 2019). To our knowledge, there are few reports on the mineral composition and their concentration of *Helichrysum* species. A previous study on *Helichrysum bracteatum* determined the presence of 15 minerals and their quantitative contents (Moskalenko & Popova, 2018). In a recent study, mineral contents of *Helichrysum odoratissimum* was examined and the result was that the plant was considered as a rich source of Na, Mg, K, Ca, P, and Fe (Afuape et. al., 2022). There is great interest in the genus Helichrysum, but little is known about *Helichrysum stoechas* and no reports are available on its mineral contents. The objective of the present works was to study the phytochemical screening, total phenolic compounds and minerals contents of *Helichrysum stoechas* collected from Libya. The plant extracts were also subjected to investigate the antioxidant, and antimicrobial properties.

2. Materials and methods

2.1. Plant materials. *Helichrysum stoechas* was freshly collected (spring 2021) from Al-Himadah region as shown in Figure 1. Leaves and stems of *Helichrysum stoechas* (Figure 2) were thoroughly washed, dried in open air, and subsequently powdered. After, the powders were stored in dark glass bottles for further uses.

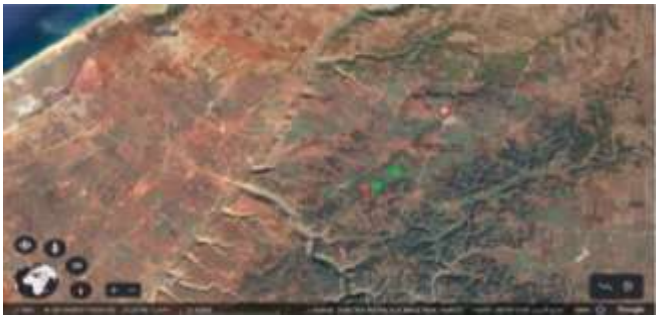

Figure 1. Al- Himadah region of *Helichrysum stoechas* location.

Figure 2. *Helichrysum stoechas*.

2.2. Preparation of extracts. The ethanol and aqueous extracts of each sample were prepared by soaking 10 g dried powder samples in 200 mL of analytical-grade ethanol and distilled water at room temperature. After 24 h, the extracts were filtered through Whatman No. 1 filter paper and residues were re-extracted under the same conditions. The filtrates were then concentrated under vacuum at 40 °C using a rotary evaporator. All the extracts were stored in sterile sample bottles at 4 °C for further experiments**.**

2.3. Phytochemical Screening. The ethanol and aqueous extracts were subjected to the screening of various phytochemical constituents, which may be present in the studied plant extracts (Kausar et al., 2021; Khuda et al., 2022; Jyothiprabha & Venkatachalam, 2016).

2.3.1. Screening for Alkaloids (Mayer's Test): 2 mL of the extract was treated with concentration hydrochloric acid (2 mL), followed by the addition of few drops of Mayer's reagent A white color precipitate produced immediately confirmed the presence of alkaloids**.**

2.3.2. Screening for Carbohydrate (Benedict's Test): 1 mL Benedict's reagent was added to 1mL of extract and the resulted mixture was boiled in water bath for 2 min. A green color formed which indicated the presence of reducing sugar.

2.3.3. Screening for Glycosides (Keller Kilianin Test): 1 mL of the extract was added to 1 mL of glacial acetic acid, followed by adding few drops of ferric chloride solution and 0.5 mL of concentrated sulfuric acid. The appearance of a reddish-brown ring at the interface confirmed the presence of glycosides.

2.3.4. Screening for Flavonoids (Alkaline Reagent Test): 2 mL of extract was treated with few drops of 20% sodium hydroxide solution. The formation of intense yellow color, which becomes colorless on the addition of dilute hydrochloric acid, indicated the presence of flavonoids.

2.3.5. Screening for Saponins (Foam Test): In a test tube, 2 mL of the extract and 6 mL of distilled water were shaken vigorously for 5 min. The formation of a persistent foam at the top of the tube indicted the presence of saponins.

2.3.6. Screening for Tannins: 1-2 drops of 1% lead acetate was added to 2 mL of the extract. A yellowish precipitate confirmed the presence of tannins.

2.3.7. Screening for Anthraquinones: 2-3 drops of 2% hydrochloric acid were added to 1 mL of extract. The formation of red precipitation confirmed the presence of anthraquinones.

2.4. Determination of Total phenolic Compounds. The Folin-Ciocalteu method was used for total phenolics determination using gallic acid as a reference (Singleton et al., 1999). Briefly, to 1.0 mL of ethanolic extract (two replicates), 1.0 mL of Folin-Ciocalteu reagent and 0.8 mL of sodium carbonate (7.5%) were added. The mixtures were incubated at 30 °C for 1.5 h. The absorbance of all samples was measured at 765 nm using the Shimadzu UV-Vis spectrophotometer. The results were expressed as ppm.

2.5. Determination of antioxidant capacity by Prussian blue method. 1 g of the powdered sample was defatted with petroleum ether. The defatted powder was subjected to sequential extraction using 10 mL of methanol twice, and then 10 mL of 1% hydrochloric acid/methanol (1/1,v/v). The extracts were evaporated under vacuum and the residue was dissolved in 10 mL methanol. 0.5 mL of the methanolic extract was diluted with 3 mL distilled water. After, 3 mL

(0.008 M) of $K_3Fe(CN)_6$, 3 mL 0.1 M HCl and 1 mL 1% $FeCl_3$ were added respectively. The absorbance of the resulting mixture (blue color) was measured at 720 nm (Wangensteen & Malterud, 2004) in central lab of Faculty of Science, Omar Al-Mukhtar University**.**

2.6. Minerals Analysis. Copper, iron and nickel in plant samples were analyzed using atomic absorption spectrophotometer (Perkin Elmer 800) and sodium, potassium and calcium contents were measured by a Flame Photometer (Jenway) according to previously described methods (Lorenz et al., 1980; Jackson, 1958) in central lab of Faculty of Science, Omar Al-Mukhtar University, Libya.

2.7. Antibacterial and antifungal activities. The microorganisms strains used in this study were Gram-positive bacteria (*Staphylococcus aurous*, *Enterococcus spp.*) and Gram-negative bacteria (*Klebsiella pneumoniae* and *Escherichia coli*), and fungus *(Candida spp.*). All the strains used were provided by Faculty of Medicine, Omar El-Mukhtar University. The agar well diffusion method was employed for the determination of antimicrobial tests of extracts. Mueller-Hinton ager plates were swabbed with the used pathogenic microorganisms. About 100 μL of ethanolic extracts were applied to the wells and the agar plates were then incubated at 37°C for 18 -24 h. The antimicrobial activity was expressed by the diameter of inhibition zones and recorded in millimeters. (Jahangirian et al., 2013).

3. Results and Discussions

In the present study, qualitative phytochemical tests were performed to detect phytochemicals in the ethanol and water extracts. The total phenol constitution and antioxidant properties were then evaluated. Next, the antibacterial and antifungal activities were characterized using four different bacterial strains and one fungi strain. For the first time, a mineral analysis of leaves and stems was carried out.

3.1. Phytochemical Screening. The results of phytochemical are presented in Table 1. Based on the screening, both extracts were found to possess tannins, alkaloids, flavonoids, and glycosides in high and moderate amounts except tannins in the aqueous stems extract and alkaloids and flavonoids in the ethanolic leaves extract were present in low amounts. Anthraquinones were only detected in the ethanolic steams extract whereas saponins were only detected in the aqueous leaves extract. The presence of these secondary metabolites possibly explains the various uses of the plant in the traditional medicine.

Table 1. Phytochemical screening of aqueous and ethanolic extracts of *Helichrysum stoechas*.

Phytochemicals	***Helichrysum stoechas***			
	aqueous extracts		ethanolic extracts	
	leaves	stems	leaves	stems
Tannins	+++	+	+++	+++
Alkaloids	+++	++	+	++
Flavonoids	+++	++	+	+++
Glycosides	+++	++	+++	++

Anthraquinones	-	-	-	+++
Saponins	+	-	-	-

+: low amount; ++: moderate amount; +++: high amount; - : absent

3.2. Total phenolic and antioxidant properties. The total phenolic compounds content of leaves and stems and antioxidant properties of ethanolic plant extracts are reported in Table 2. The findings showed that The highest levels of total phenolic content and antioxidant activity were found in the leaves of *Helichrysum stoechas* (12.6 ppm and 8.48 ppm respectively), while the lowest levels were in the stems of *Helichrysum stoechas* (11.9 ppm and 7.28 ppm respectively). The both extracts had appreciable antioxidant activity and similar results have been reported by others in different values. (Haddouchi et al., 2014; Carini et al., 2001; Albayrak, et al., 2010). Many reports have indicated a linear correlation of the total phenolic content and antioxidant capacity (Haddouchi et al., 2014; Ha et al., 2020) and our results (Table 2) are in agreement with those reports.

Table 2. Total phenolic compound content (ppm) and antioxidant activity (ppm) in ethanolic extract of *Helichrysum stoechas*.

Helichrysum stoechas	**leaves**	**stems**
Total Phenolics (ppm)	12.6	11.9
Antioxidant Activity (ppm)	8.48	7.28

3.3. Antibacterial and antifungal activities. It is well known that the presence of phytochemicals such as tannins, alkaloids, flavonoids and glycosides are the principal reasons for a plant to exhibit antimicrobial activities (Nethathe et al., 2011). The antimicrobial activities of Libyan *Helichrysum stoechas* have been studied by different researchers. A report by Ibrahim et al. (2017) exhibited potential antibacterial effects against *Staphylococcus aureus* and *Escherichia coli* of the methanolic extract of *Helichrysum stoechas* collected from Hanya region, Libya. Additionally, the antimicrobial activity of ethanol extracts from aerial parts of the plant, collected from Al-Jabal Al-Akhdar-Libya, was investigated in a medicinal plants lab, Benha University, Egypt. Their findings were that the extract was active against *Staphylococcus aureus* and *Escherichiacoli*, but inactive against *Candida albicans and Candida tropicalis* (Sobhy et al., 2007). More recently, the aqueous extract of the leaves of *Helichrysum stoechas*, collected from Al-Jabal Al-Akhdar region, Libya, was reported to possess antifungal properties against *Sclerotinia sclerotiorum* (Hypa & El-Gali, 2022).

In the present study, the antimicrobial activities of leaves and stems extracts of *Helichrysum stoechas* were investigated according to their inhibition zones against the selected microorganisms strains (Table 3). The ethanolic extract of the leaves showed considerable antibacterial activity against *Escherichia coli* with an inhibitory zone (83 mm), while the ethanolic extract of the stems showed anticandida activity with an inhibitory zone (7 mm).

Table 3. The antibacterial and antifungal activities (mm) of ethanolic extracts of leaves and stems of *Helichrysum stoechas*.

	Helichrysum stoechas		
Test microorganisms	stems	leaves	control
Staphylococcus aureus	2	1	22
Enterococcus spp.	-	2	23
Klebsiella pneumoniae	-	-	81
Escherichia coli	1	83	81
Candida spp.	7	2	3

The results mentioned above may be disagree with previous studies of *Helichrysum stoechas*, collected from Libya, in some data. Differences in the chemical composition and phenolic contents of *Helichrysum stoechas*, and consequently, in its biological properties such as antioxidant and antimicrobial properties, may be due to differences in the collection area, climatic condition, storage, and processing methods. For example, the total phenolic content of the methanolic extract of aerial parts of *Helichrysum stoechas* from Hanya region in summer was 8.07 mg gallic acid equivalents/g (Ibrahim et al., 2017) while we estimated it to be 12 ppm.

3.4. Mineral Composition. Since minerals are essential for metabolic functions, mineral analysis of plants is important in order to use them as a source of minerals (Huskisson et al., 2007; Afuape, 2022). Macro (sodium, potassium, and calcium) and micro (iron, copper, and nickel) minerals contents of the leaves and stems of *Helichrysum stoechas* were analyzed (Table 4). The data showed that sodium and potassium were in high levels in both leaves and stems, though higher in the leaves than in the stems, exceeding 50 ppm except for potassium content in the stems (11 ppm). The calcium content was significantly the same in stems and leaves (11 ppm), while the contents of the other studied elements (Fe, Cu, and Ni) were less than 3 ppm. As a result, the researchers suggest that the aerial part of the studied plant is a rich source of macro-mineral composition (Na, K and Ca). It is a well-known fact that, Na in conjunction with K play an important role in the maintenance of normal levels of fluid inside the cells, and Ca is essential for bone growth and muscle contraction. Together, Fe and Cu are required in formation of red blood cells (Huskisson et al., 2007; Ali, 2023).

Table 4. The elements content (ppm) in *Helichrysum stoechas* parts.

Elements	***Helichrysum stoechas***	
	leaves	stems
Na	84	52
K	76	11
Ca	11	11
Fe	2.68	2.82
Cu	1.40	1.36
Ni	1.29	1.27

Conclusions

Our results allow to conclude that the tested parts of *Helichrysum stoechas* are containing various secondary compounds like alkaloid, tannins, flavonoids, anthraquinones, saponins and glycosides. In addition, the plant contains a considerable amount of phenolic, and possesses interesting antioxidant and antimicrobial properties. The results reported here can be considered as the first information of the mineral profile of *Helichrysum stoechas*.

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