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Phytochemical Content, Antioxidant Activity, Essential Oils and Antibacterial Potential of Egyptian *Phlomis floccosa* D. Don and *Glebionis coronaria* (L.) Cass. ex Spach

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ABSTRACT

Wild medicinal plants have gain attention due to their active secondary metabolites that possess distinctive therapeutic and pharmacological properties. This study focused on estimating the phytochemical content of *Phlomis floccosa* D. Don and *Glebionis coronaria* (L.) Cass. ex Spach, two medicinal plant species collected from Marsa Matrouh City in Egypt. Water, methanol, and petroleum ether extracts were prepared from both species for antioxidant activity assessment using the DPPH assay method. Additionally, Gas Chromatography-Mass Spectrometry was utilized to identify essential oils present in the aerial part of both species while antibacterial activity against nine isolates was also evaluated. Our results revealed that water extracts of *P. floccosa* and *G.coronaria* contained higher amounts of phenols, flavonoids, tannins, alkaloids, and saponins than other extract types tested. In terms of essential oil components, *P. floccosa*'s aqueous extraction yielded 19 oxygenated as well as non-oxygenated hydrocarbon compounds; whereas, *G. coronaria's* extracted oil contained forty-six volatile compounds including 17 hydrocarbons, five terpenes, eighteen fatty acids/lipids, six steroids etc., respectively. Finally, in relation to antimicrobial effects observed: *Bacillus subtilis* and *Staphylococcus aureus* were inhibited by water extract of *P. floccosa*; on the other hand, *G. coronaria* exhibited inhibitory effects against *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *S. epidermidis* by the use of its aqueous or methanolic solvent systems. Generally, it can be concluded that these plant species may provide promising sources of natural products with antioxidant and antibacterial activities

Keywords: *Chrysanthemum*; GC-MS; Medicinal Plants; *Phlomis*, Phytochemistry.

INTRODUCTION

Numerous pharmacological and therapeutic properties have been demonstrated in herbs, spices, and plants. Recently, the majority of researches have been focused on isolating and identifying the active compounds found in these plants and herbs. Many studies have examined the potential of their secondary metabolites, such as phenols, flavonoids, tannins, etc. as antioxidants against reactive oxygen species (ROS), which are the cause of a number of diseases such as cancer, diabetes, atherosclerosis, heart diseases, and other free radicals induced diseases (Broadhurst *et al.*, 2000). Moreover, numerous studies on the antimicrobial and herbicidal properties of plant metabolites have suggested the potential application of plant natural products for the synthesis of herbicides and antimicrobial agents (Chon *et al.*, 2005; Amoo *et al.*, 2008).

Glebionis coronaria (L.) Cass. ex Spach (synonym: *Chrysanthemum coronarium* L.) is an annual plant belonging to family Asteraceae (Compositae) and universally known as Chrysanthemum, Pyrethrum or Tango. It is mainly considered as an important medicinal plant but also used as an ornamental plant (Sulas *et al.*, 1999). It is a herbaceous plant with an aromatic flavor, which growth as troublesome weed among cereals crops, with stems up to 100 cm tall. Leaves are oblong, frequently with two-part florets with a large head, usually white and yellow. It is available in tropical and subtropical regions (Prakash & Rao, 2018) and broadly distributed in Japan, China, Philippines and the Mediterranean region (Basta *et al.*, 2007). Because of numerous volatile components in their essential oils and aromatic value, many of *Chrysanthemum* plants are used in the flavoring and perfume production. Flower heads of *G. coronaria* are commonly consumed as chamomile adulterants. The leaves are used in Japan to reduce unpleasant odors in foods (da Silva, 2004). *Glebionis coronaria* has been used in traditional Egyptian medicine (Tackholm and Boulos, 1974). The essential oil of *G. coronaria* exhibited antimicrobial actions and the water extract of flower heads have phytotoxic impacts (Alvarez-Castellanos *et al.*, 2001; Hosni *et al.*, 2013). In Japan, the stems and leaves which are rich in minerals, vitamins and β-carotenoids are consumed by farm animals as forage and also used to reduce fishy flavors in prepared foods (Kasahara and Nishibori, 1995; Sulas and Caredda, 1997). Several compounds with high biological values have been isolated from *G. coronaria* (Chuda *et al.*, 1996; Chuda *et al.*, 1998; Takenaka *et al.*, 2000).

On the other hand, *Phlomis* is an important medicinal genus of perennial herbs belonging to Lamiaceae (Labiatae). It includes approximately 100 species spread in Europe, Asia and Africa (El-Sadek *et al.*, 2017). In Egypt, there are two species of the genus *Phlomis*: *P. aurea* and *P. floccosa* (Khedr *et al.*, 2020). *Phlomis* leaves are entire, opposite petiolate, veined, wrinkled, or reticulated. The flowers are assembled in spiral patterns around the stems. *Phlomis* spp. is valuable sources of several glycosides, including diterpenoids, iridoids, phenylethanoids and flavonoids (Sarkhail *et al.*, 2005). Species of this genus have also been applied as tonics, healings and stimulants (Limem *et al.*, 2011). Some biological and pharmacologic activities have been recognized as well such as antidiabetic (Sarkhail *et al.*, 2007), antimicrobial (Wafa *et al.*, 2016), anti-inflammatory (Shang *et al.*, 2011),

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antifebrile and immuno-oppressive (AbdEIslam *et al.*, 2013). *Phlomis floccosa* D. Don is a perennial plant, growing to 40 cm and native to Egypt, Libya and Tunisia. In Egypt, this plant inhabits rocky habitats along the Western Mediterranean region (Boulos, 2002). In Tunisia, the essential oil isolated from *P. floccosa* was found to exhibit significant biological activities (El Mokni *et al.*, 2019).

The objectives of this work were to: (i) evaluate phytocompounds in the water, methanol, and petroleum ether extracts of *Phlomis floccosa* and *Glebionis coronaria*, (ii) determine the antioxidant properties of different extracts of both species, (iii) study the essential oils extracted from the shoots of both species, and (iv) evaluate the antibacterial activity of both species against nine strains.

MATERIALS AND METHODS

Study area and plant sampling

The study was carried out in Marsa Matrouh City, which is located along the Mediterranean Sea in Egypt. The city is known for its diverse flora, including many wild medicinal plant species with potential therapeutic properties. In this study, aerial parts of *Phlomis floccosa* and *Glebionis coronaria* were collected from different habitats within Marsa Matrouh to investigate their phytochemical content and potential medicinal uses. Specifically, *P. floccosa* was obtained from a wadi-bed microhabitat situated along Wadi Habis to the west of Marsa Matrouh at coordinates 31^{o} 22.86 N and 27^{o} 02.228 E; whereas *G.coronaria* was found on roadside habitat within the city at coordinates 31^{o}21.36N and 27^{o}08.996E.

Samples were harvested during flowering stage of these species in the year 2021, air-dried naturally until complete dryness before being ground into powder form for further analysis purposes. Finally, the dried plant material was safely stored within polythene bags for future use.

Preparation of plant extracts

Water extracts were obtained by adding 10 grams of dried plant material to 100 ml distilled water in a conical flask, which was was kept in water bath shaker at 200 rpm with 70^{o}C for 30 minutes. The mixture was filtered, using a Buchner funnels and Whatman filter papers No. 1, and then stored at 5^{o}C. Methanol extracts were obtained using the same process but with reduced temperature (40^{o}C) and longer extraction time (4 hours). Petroleum ether extracts were obtained through Soxhlet extraction using 10 grams of dried plants and 200 ml petroleum ether, heated for six hours until complete extraction (Redfern *et al.*, 2014). The resulting extract was condensed to half its original volume, filtered, and stored at 5^{o}C.

Phytochemical analysis

Total phenolic content

The test was conducted on the plants extracts to determine their total phenolic content. Folin-Ciocalteu assay was used following the procedure informed by Wolfe *et al.* (2003), and Issa *et al.* (2016), where the characteristic values were calculated as milligram gallic acid equivalents/grams of the dried plant using the standard curve of gallic acid.

Flavonoids content

The test was conducted using the aluminum chloride colorimetric assay of the plants extracts described by Zhishen *et al.* (1999), with the standard curve of catechin. The amount of flavonoids is expressed as milligrams of catechin equivalent/ gram of plant's dry weight.

Tannins content

The vanillin-hydrochloride test (Burlingame, 2000), which measures the sample's absorbance following treatment with freshly generated vanillin-hydrochloride, was used to assess the tannin concentration. The tannin content of the plants extracts was measured and expressed as grams of tannic acid equivalents/ 100 grams of dried plant. The standard curve for tannic acid was used to calculate the amount of tannins present in the investigated samples.

Alkaloids content

Using an ammonium hydroxide solution, the total alkaloid content was calculated. One milliliter of the plant sample and one milliliter of a concentrated ammonium hydroxide solution were combined. The solution was kept to settle until the development of a precipitate which was then filtered using previously weighted filter paper. 2 ml of a diluted ammonium hydroxide solution were used for washing. The weighed filter paper was dried with the precipitate after the filtration procedure, and then weighed once more with the precipitate. As previously reported by Harborne, the alkaloid contents were estimated as a weighted value (Harborne, 1998).

Saponins content

Using the technique of Obadoni and Ochuko (2002), saponins content was determined. In this procedure, a weighted dry plant sample was added to 100 mL of the preferred extraction solvent. For two hours at 200 rpm and 30°C, the solution was shaken. Using filter paper, the prepared extract was filtered out, and the leftover plant material was then once again extracted using the extraction solvent and filtered. Over a water bath, the produced extract was concentrated to a set volume (40 ml). Therefore, 40 ml of the extracted sample were placed into a separate funnel, followed by 20 ml of diethyl ether, and mixture was vigorously agitated. The organic-ether layer was separated from the aqueous layer. The separation process was repeated multiple times. The aqueous layer was then treated twice with sodium chloride solution (10 ml, 5%) before being treated once with n-butanol (60 ml). To evaporate the solvent, the residual solution was heated. The samples were oven-dried and weighed after the solvent had completely evaporated. The saponins content was expressed as mg of saponins/ g of dry extract.

Antioxidant activity using the 2, 2-diphenyl-hydrazyl-hydrate (DPPH) assay

The antioxidant capacity of the plant samples was tested by the DPPH$^{\bullet}$ colorimetric method with ascorbic

acid as a standard, as described by Kitts *et al.* (2000). The samples were mixed with an equivalent amount of solvents (water, methanol and petroleum ether) to create a serial dilution. A 0.135 mM DPPH solution was made, and an equivalent volume of it was added to each sample. After the addition of the DPPH solution, the samples were retained in the dark for 30 minutes. The absorbance was measured at 517 nm. The remaining DPPH was estimated by stratifying the following equation:

DPPH scavenging activity (%) =

$$(A_0 - A_1) / A_0 \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

The effective concentration, or IC50, was calculated by plotting the values of % DPPH remaining against sample concentrations in mg/ml. The IC50 value represents how many antioxidants are required to reduce the concentration of DPPH solution by 50%. Lower IC50 values indicate higher antioxidant capacity for the tested samples. As reported by Parejo *et al.* (2000), there is an inverse correlation between the tested sample's antioxidant ability and their respective IC50 values. Therefore, lower IC50 values reflect higher antioxidant activity in the tested samples.

Essential oil analysis

The Gas Chromatography–Mass Spectrometry (GC-MS) analysis of the essential oil from the aerial parts of two plant species was processed using a TRACE GC-Ultra Gas Chromatograph (THERMO-Scientific Corp., USA), joined with a THERMO-mass spectrometer-detector (Wesołowska *et al.*, 2015). The GC-MS system was provided with a TG-5 MS column. Helium was used as a carrier gas at a flow rate of 1.0 ml/min and a split ratio of 1:10 using the next temperature practice: 60°C for 1 min; rising at the rate of 3.0°C/min. till 240°C and held for 1 min. The injector and detector were adjusted at 240°C. Diluted samples (1:10 hexane, v/v) of 0.2 µL of the mixtures were injected. Mass spectra were taken by electron ionization using a m/z 40–450 spectral range. The majority of compounds were identified using mass-spectrometry (authentic-chemicals, NSIT library and Wiley-spectral library).

Antibacterial activity

The tested extracts were evaluated against nine bacterial human pathogens, namely *Staphylococcus aureus, Bacillus subtilis, Enterobacter cloacae, Listeria monocytogenes, Salmonella typhi, Bacillus cereus, Staphylococcus epidermidis, Escherichia coli* and *Pseudomonas aeruginosa*. The agar well diffusion technique was employed to evaluate the antimicrobial activity of the extracts (Prabuseenivasan *et al.*, 2006). In this method, a specific volume of inoculum was spread over the surface of an agar plate. Then sterile cork-borer or tip was used to create 7 mm-diameter holes in which 40 µL of tested extract at desired concentrations were added. After adding the samples into wells on plates containing different strains separately; each plate was incubated under appropriate conditions according to test bacteria growth requirements. Finally, the diameter of inhibitory zones around each well was measured in millimeters and recorded as average results for multiple tests performed

Statistical analysis

The values performed in this study were triplicated and expressed as mean± standard error. The significance was evaluated by XLSTAT (version 2016) using Kruskal-Wallis one-way ANOVA. Differences at $p \leq 0.05$ were counted significant.

RESULTS

Phytochemical analysis of *P. floccosa* and *G. coronaria*

The concentrations of phenols, flavonoids, tannins, alkaloids and saponins in the different types of *P. floccosa* and *G. coronaria extracts* are summarized in Table 1. In terms of extract types (water, methanol and petroleum ether), *P. flocossa* and *G. coronaria* showed significant differences ($p \leq 0.05$) for the estimated phytochemical contents. Water extracts of *P. floccosa* and *G. coronaria* showed higher phenols, flavonoids, tannins, alkaloids and saponins than other extract types. On the other hand, petroleum ether extracts exhibited the least phytochemical contents in both plants. Water extracts had the highest phenolic levels with concentrations of 312.02 and 260.57 mg gallic acid g^{-1} dry extract for *P. floccosa* and *G. coronaria*, respectively. Petroleum ether extracts exhibited the lowest phenolic levels for both species, where petroleum ether extract of *P. floccosa* recorded the lowest phenols content concentration (7.59 mg gallic acid g^{-1} dry extract).

The flavonoid content of *G. coronaria* and *P. floccosa* water extracts was found to be the highest among all the tested plant extracts, with concentrations of 87.83 mg catechin g^{-1} dry extract and 49.40 mg catechin g^{-1} dry extract, respectively. On the other hand, petroleum ether extracts showed lower tannin contents compared to water extracts, with concentrations of 5.82 mg tannic acid g^{-1} dry extract for *G. coronaria* and 1.11 mg tannic acid g^{-1} dry extract for *P. floccosa* being recorded as the lowest values in both plants.

Interestingly, tannin levels were much higher in water extracts than those obtained from methanol or petroleum ether extractions; the maximum content was observed in *P. floccosa* at 45.41 mg tannic acid g^{-1} dry extract followed by *G. coronaria* at 49.76 mg tannic acid g^{-1} dry extract. Furthermore, alkaloid content was also found to be higher in water extracts of both plants compared to other solvents used during extraction processes; *P. floccose* and *G. coronaria* had alkaloid contents of 17.59 mg/g and13 .89 mg/g respectively.

Total saponins were also significantly higher in water-based extractions compared to methanolic ones; *P. floccosa* recorded highest total saponin concentration out of all samples tested at 11 .58 mgg^{-1} while that value was 9.76 mg/g for *G. coronaria's* aqueous

extraction followed by its methanolic extract which contained 5.97 $mgg^{-1}$of total saponins.

Antioxidant activity

Table (2) summarizes the DPPH scavenging activity of extracts derived from both plant species. Water and methanol extracts exhibited higher antioxidant activeities than petroleum ether extract in both cases. The highest scavenging activity was observed in the water extracts, with IC50 values of 0.005 ±0.0001 mg/mL for *P.floccosa* and 0.002± 0.0002 mg/mL for *G.coronaria*. Methanolic extracts also displayed significant levels of scavenging activity, with comparable IC50 values of 0.025 ±0.001 mg/mL (*P. floccosa*) and 0.071± 0.002 mg/mL (*G.coronaria*). In contrast, petroleum ether extractions showed lower radical scavenging potential compared to other solvent systems employed during extraction processes across both plants' samples tested.

These results suggest that aqueous or methanolic solvents are more effective at extracting bioactive compounds with potent antioxidant properties from leaves or other parts of *P. floccosa and G. coronaria* plants than petroleum ether extract method.

Table (1): Phytochemical analysis of different extracts from *P. floccosa* and *G. coronaria*

Solvent used	Plant species	Major phytochemical ccompounds detected				
		Phenolics (mg gallic acid g^{-1} dry extract)	Flavonoids (mg catechin g^{-1} dry extract)	Tannins (mg tannic acid g^{-1} dry extract)	Alkaloids (mg g^{-1} dry extract)	Saponins (mg g^{-1} dry extract)
Water	*P. floccosa*	312.02 ±9.01^{b}	49.40 ±1.71^{b}	45.41 ±1.05^{a}	17.59 ±0.41^{b}	11.58 ±0.27^{b}
	G. coronaria	260.57 ±7.52^{b}	87.83 ±1.10^{b}	49.76 ±1.04^{a}	13.89 ±0.32^{b}	9.76 ±0.23^{b}
Methanol	*P. floccosa*	160.19 ±3.70^{ab}	42.93 ±0.99^{ab}	36.61 ±1.06^{ab}	6.98 ±0.20^{ab}	3.68 ±0.11^{ab}
	G. coronaria	190.33 ±4.40^{ab}	31.82 ±2.03^{ab}	45.11 ±1.44^{ab}	8.35 ±0.24^{ab}	5.97 ±0.17^{ab}
Petroleum ether	*P. floccosa*	7.59 ±0.37^{a}	1.39 ±0.04^{a}	1.11 ±0.04^{a}	0.86 ±0.03^{a}	0.74 ±0.03^{a}
	G. coronaria	34.21 ±1.19^{a}	11.15 ±0.32^{b}	5.82 ±0.24^{a}	1.50 ±0.06^{a}	1.29 ±0.05^{a}

Data are represented in mean ±SE. Means with different letter per column, for the same measured compound per plant species, are significant different at $p \leq 0.05$.

Table (2): The antioxidant results of the studied plant extracts as compared with the natural antioxidant ascorbic acid.

Solvent used	Plant species	Concentration used (mg/ml)	% Residual DPPH	Scavenging activity (%)	IC50 (mg/ml)
Water	*P. floccosa*	0.0210	33.05±0.95	66.95 ±1.93	0.005 ±0.0001
		0.0110	40.72±1.18	59.28 ±1.71	
		0.0050	50.09±1.45	49.91 ±1.44	
		0.0030	55.54±1.60	44.46 ±1.28	
	G. coronaria	0.0030	37.14±1.07	62.86 ±1.81	0.002 ±0.0002
		0.0020	59.11±1.71	40.89 ±1.18	
		0.0009	92.84±2.68	7.16 ±0.21	
		0.0004	96.42±2.78	3.58 ±0.10	
Methanol	*P. floccosa*	0.0440	23.03±0.66	76.97 ±2.22	0.025 ±0.001
		0.0220	66.51±1.54	33.49 ±0.97	
		0.0110	83.25±2.88	16.75 ±0.48	
		0.0060	99.52±3.45	0.48 ±0.01	
	G. coronaria	0.1400	20.61±0.48	79.39 ±2.29	0.071 ±0.002
		0.0700	54.59±1.58	45.41 ±1.31	
		0.0350	81.64±1.89	18.36 ±0.53	
		0.0180	89.69±2.59	10.31 ±0.30	
Petroleum ether	*P. floccosa*	1.949	41.56±1.07	36.42 ±1.05	6.508 ±0.188
		0.974	46.55±2.35	18.65 ±0.54	
		0.487	63.73±1.84	9.55 ±0.28	
		0.244	81.64±2.35	0.44 ±0.01	
	G. coronaria	8.555	25.70±0.74	74.30 ±2.14	0.506 ±0.015
		4.278	44.20±1.28	55.80 ±1.61	
		2.139	67.84±1.96	32.16 ±0.93	
		1.069	90.01±2.60	9.99 ±0.29	
Positive control	**Ascorbic acid**	0.062	15.267±0.44	85.19 ±2.46	0.022 ±0.001
		0.031	39.084±1.13	62.07 ±1.79	
		0.016	61.069±1.76	40.74 ±1.18	
		0.008	74.809±2.16	27.41 ±0.79	

Essential oils of *P. floccosa* and *G. coronaria*

The GC-MS chromatogram of the key components of the essential oils of *P. floccosa* is shown in Figures (1) and (2). The results in Table (3) indicate that, forty volatile components were detected in *P. floccosa* with 100.02% of the total area percentages during 49.09 min. Nonacosane (12.77%), oleic acid (12.58%), lup-20(29)-ene-3, 28-diol (6.59%), lup-20(29)-en-3-ol, (3)-(6.36%), dotriacontane (6.21%), and ethyl 3,7,12-trihydroxycholan-24-oate (5.76%) were identified as the major components. The results also indicated 19 components are related to oxygenated and non-oxygenated hydrocarbons with the major abundance for nonacosane (12.77%). Furthermore, three components are related to the terpene class with the major abundance for 3,7,11-trimethyldodecan-1-ol (1.69%), while twelve components of the fatty acids and lipids category were identified with the major abundance for oleic acid (12.58%), and 2,3-bis ((trimethylsilyl)oxy) propyl (9Z, 12Z)-octadeca-9, 12-dienoate (4.34%). The steroid category included six volatile components with a majority of the abundance for Lup-20 (29)-ene-3,28-diol (6.59%), Lup-20(29)-en-3-ol, (3α)- (6.36%), and ethyl 3,7,12-trihydroxycholan-24-oate (5.76%). On the other hand, the GC-MS of the key volatile components in *G. coronaria is* shown in Figures (3) and (4). The results in Table (4) indicated that forty-six volatile components accounted for 100.02% of the total area percentages during 48.88 min. The most abundant components are interpreted as oleic acid (14.96%), heptacosane (8.27%), 2-methylhexadecan-1-ol (7.21%), nonacosane (6.55%), methyl (E)-octadec-11-enoate (4.42%), dotriacontane (3.8%), Lup-20(29)-en-3-ol, (3α)- (3.57%), palmitic acid (3.55%), 2,6,10-trimethyltetradecane (3.31%), bis (6-methylheptyl) phthalate (3.24%), docosane (3.22%), 3,7,11-trimethy-ldodecan-1-ol (3.2%), and methyl palmitate (3.09%). The hydrocarbons category compromised 17 volatile components, five components are related to terpenes, 18 components are related to fatty acids and lipids; while steroidal components compromised six volatile components.

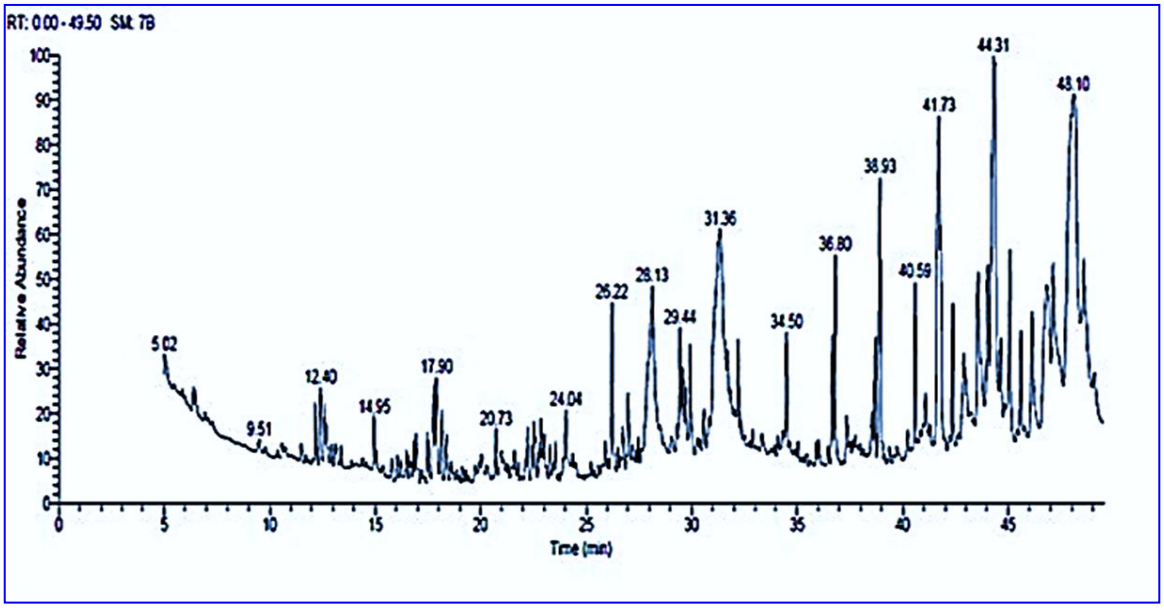

Figure (1): GC–MS chromatogram of essential oil components from the aerial parts of *P. floccosa*.

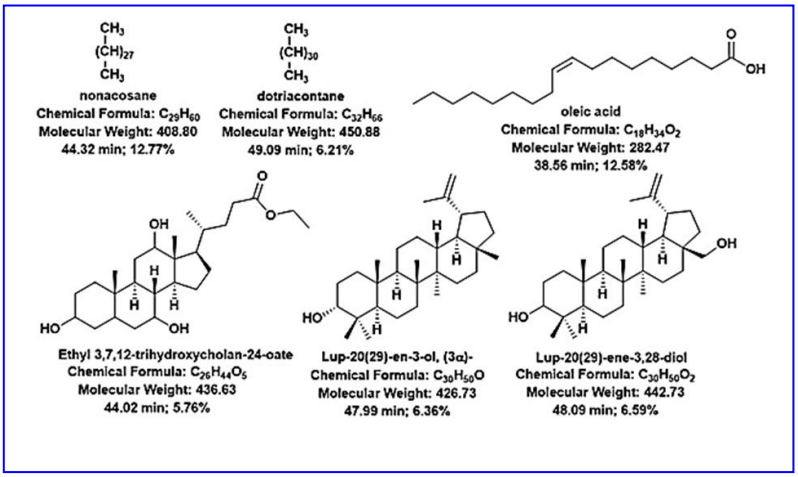

Figure (2): Molecular structures of the major abundant volatile components of *P. floccosa*.

Table (3): Phytochemical composition of essential oils extracted from aerial parts of *P. floccose* using gas chromatography/mass spectrometry (GC/MS) analysis.

Sample number	Chemical name	Classification	Retention time (RT, min)	Molecular weight	Molecular formula	Area %
Hydrocarbons						
1	2,6-dimethylheptan-1-ol	Hydrocarbon	5.03	144.26	$C_9H_{20}O$	0.41
2	8-methylenepentadecane		6.37	224.43	$C_{16}H_{32}$	0.21
3	dec-1-ene		6.47	140.27	$C_{10}H_{20}$	0.26
4	2-methyldecan-1-ol		12.18	172.31	$C_{11}H_{24}O$	1.5
5	Tetradecane		12.69	198.39	$C_{14}H_{30}$	0.44
6	Dodecane		12.93	170.34	$C_{12}H_{26}$	0.78
7	2,3,5,8-tetramethyldecane		16.04	198.39	$C_{14}H_{30}$	0.28
8	Tetradecan-1-ol		16.23	214.39	$C_{14}H_{30}O$	1.41
9	1-allyl-1-methyloctahy-dronaphthalen-2(1*H*)-one		17.77	206.33	$C_{14}H_{22}O$	1.47
10	2,6,10-trimethyltetr-adecane		17.90	240.48	$C_{17}H_{36}$	2.63
11	(2*E*, 4*E*, 6*E*, 8*E*)-3,7-dimethyl-9-(2, 6, 6-trimethylcyclohex-1-en-1-yl) nona-2, 4, 6, 8-tetraenal (Retinal "Vitamin A")		20.97	284.44	$C_{20}H_{28}O$	0.36
12	2-methylhexadecan-1-ol		22.43	256.47	$C_{17}H_{36}O$	3.56
13	docosane		22.55	310.61	$C_{22}H_{46}$	1.03
14	stearaldehyde		35.90	268.49	$C_{18}H_{36}O$	0.26
15	hexacos-1-ene		37.35	364.70	$C_{26}H_{52}$	0.64
16	heptacosane		38.93	380.75	$C_{27}H_{56}$	3.84
17	nonacosane		44.32	408.80	$C_{29}H_{60}$	12.77
18	tetratriacontane		44.38	478.93	$C_{34}H_{70}$	3.33
19	dotriacontane		49.09	450.88	$C_{32}H_{66}$	6.21
Terpenes						
20	3,7-dimethylocta-1,6-dien-3-ol	Monoterpene	13.38	154.25	$C_{10}H_{18}O$	0.22
21	(1*S*, 2*R*, 7*S*, 8*S*)-2, 6, 6-trimethyl-9-methylenetricyclo [5.4.0.0^{2,8}] undecane	Sesquiterpene	14.95	204.36	$C_{15}H_{24}$	0.55
22	3,7,11-trimethyldodecan-1-ol		17.50	228.42	$C_{15}H_{32}O$	1.69
Fatty acids and lipids						
23	methyl octadeca-6,8-diynoate	Ester of fatty acid	16.62	290.45	$C_{19}H_{30}O_2$	0.22
24	methyl 11-((2*R*, 3*S*)-3-pentyloxiran-2-yl) undecanoate		22.13	312.49	$C_{19}H_{36}O_3$	0.22
25	methyl palmitate		26.22	270.46	$C_{17}H_{34}O_2$	2.07
26	palmitic acid	Fatty acid	28.14	256.43	$C_{16}H_{32}O_2$	1.68
27	methyl (*E*)-octadec-11-enoate	Ester of fatty acid	29.58	296.50	$C_{19}H_{36}O_2$	2.68
28	methyl stearate		29.94	298.51	$C_{19}H_{38}O_2$	1.35
29	(*E*)-octadec-13-enoic acid	Fatty acid	31.07	282.47	$C_{18}H_{34}O_2$	0.22
30	(*Z*)-octadec-11-enoic acid		31.36	282.47	$C_{18}H_{34}O_2$	2.68
31	2,3-dihydroxypropyl palmitate	Ester of fatty acid	36.01	330.51	$C_{19}H_{38}O_4$	0.34
32	*bis*(6-methylheptyl) phthalate		36.80	390.56	$C_{24}H_{38}O_4$	2.53
33	oleic acid	Fatty acid	38.56	282.47	$C_{18}H_{34}O_2$	12.58
34	2,3-bis((trimethylsilyl)oxy)pro-pyl (9Z,12Z)-octadeca-9,12-dienoate	Ester of fatty acid	48.58	498.90	$C_{27}H_{54}O_4Si_2$	4.34
Steroids						
35	Ethyl 3,7,12-trihydroxycholan-24-oate	Steroid	44.02	436.63	$C_{26}H_{44}O_5$	5.76
36	9,19-Cyclolanost-24-en-3-ol,acetate, (3α)-		44.65	468.77	$C_{32}H_{52}O_2$	2.87
37	4, 4a, 6b, 8a, 11, 11, 12b, 14a-Octamethyl-3-oxodocos-ahydro-2-picenyl acetate		45.09	484.77	$C_{32}H_{52}O_3$	2.18
38	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate		46.13	498.0	$C_{33}H_{54}O_3$	1.50
39	Lup-20 (29)-en-3-ol, (3α)-		47.99	426.0	$C_{30}H_{50}O$	6.35
40	Lup-20 (29)-ene-3,28-diol		48.09	442.0	$C_{30}H_{50}O_2$	6.58
Total						**100.00**

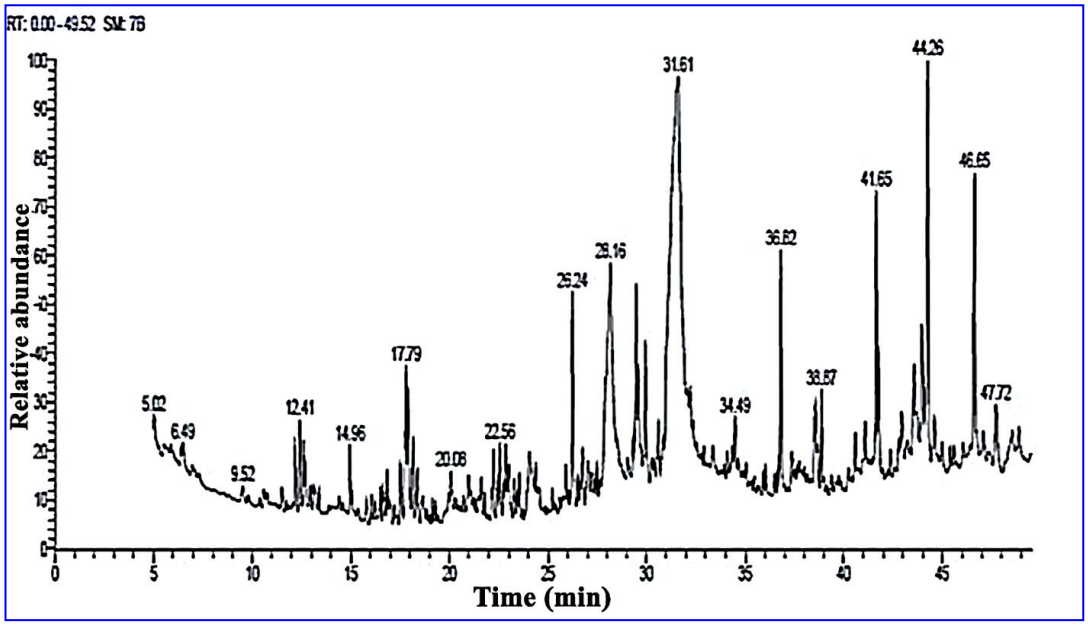

Figure (3): GC-MS chromatogram of the main volatile oils extracted from aerial parts of *Glebionis coronaria*.

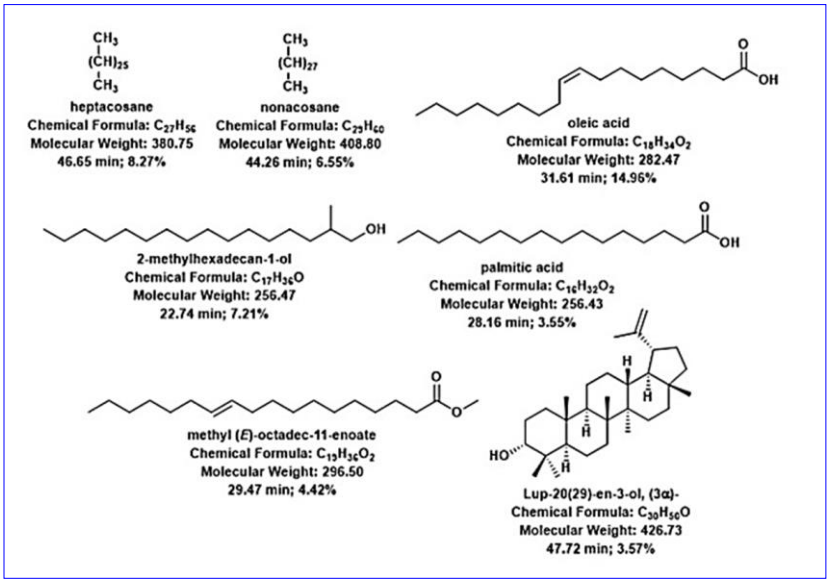

Figure 4: Molecular structures of the major abundant molecules of *Glebionis coronaria* essential oils.

Antibacterial activity

The inhibitory effect of water, methanolic and petroleum ether extracts of the two plants *P. floccosa* and *G. coronaria on* nine bacterial isolates is shown in Table (5). The results indicated that both water and methanolic extracts of *P. floccosa* and *G. coronaria inhibited* the growth of *S. aureus*. A water extract of *P. floccosa* revealed the highest antibacterial activity against *B. subtilis* (20 mm). On the other hand, all extracts of *P. floccosa* exhibited no antibacterial activity against *E. cloacae*, *L. monocytogenes*, *S. typhi*, *B. cereus*, *S. epidermidis*, *P. aeruginosa* and *E. coli*. Water and methanolic extracts of *G. coronaria* were the only extracts that inhibited the growth of *L. monocytogenes*, *B. cereus* and *S. epidermidis*. However, these extracts did not show any inhibition effect on *B. subtilis*, *E. cloacae*, *S. typhi*, *P. aeruginosa* and *E. coli*. Petroleum ether extracts of all tested species did not show any antibacterial effect on all tested bacterial strains.

DISCUSSION

Biologically active substances are typically found in small amounts in plants. According to Quispe-Condori *et al.*, (2008), a successful extraction method is one that can produce a high yield of the required extracts with little alteration to their functional properties. A good solvent is distinguished by its optimal extraction

Table (4): Phytochemical composition of essential oils extracted from aerial parts of *Glebionis coronaria* using gas chromatography/mass spectrometry (GC/MS) analysis.

Sample number	Chemical name	Classification	Retention time (RT, min)	Molecular weight	Molecular formula	Area %
Hydrocarbons						
1	1-hydroperoxyhexane	Hydrocarbon	6.49	118.18	$C_6H_{14}O_2$	0.38
2	2,6,10-trimethyltetradecane		11.51	240.48	$C_{17}H_{36}$	3.31
3	2-methyldecan-1-ol		12.19	172.31	$C_{11}H_{24}O$	2.03
4	dodecane		12.95	170.34	$C_{12}H_{26}$	0.33
5	6-methyloctadecane		15.06	268.53	$C_{19}H_{40}$	0.22
6	2-allyl-5-(*tert*-butyl)benzene-1,4-diol		17.79	206.29	$C_{13}H_{18}O_2$	2.50
7	(*E*)-1,2,3-trimethoxy-5-(prop-1-en-1-yl)benzene		18.64	208.26	$C_{12}H_{16}O_3$	0.67
8	Retinal		20.98	284.0	$C_{20}H_{28}O$	0.57
9	2-methylhexadecan-1-ol		22.74	256.47	$C_{17}H_{36}O$	7.21
10	3-(tetradecyloxy)propane-1,2-diol		30.59	288.47	$C_{17}H_{36}O_3$	0.91
11	stearaldehyde		35.92	268.49	$C_{18}H_{36}O$	0.27
12	bis(6-methylheptyl) phthalate		36.82	390.56	$C_{24}H_{38}O_4$	3.24
13	docosane		38.87	310.61	$C_{22}H_{46}$	3.22
14	nonacosane		44.26	408.80	$C_{29}H_{60}$	6.55
15	heptacosane		46.65	380.75	$C_{27}H_{56}$	8.27
16	2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-4H-chromen-4-one		48.55	344.32	$C_{18}H_{16}O_7$	1.70
17	dotriacontane		48.88	450.88	$C_{32}H_{66}$	3.8
Terpenes						
18	3,7-dimethylocta-1,6-dien-3-ol	Monoterpene	13.39	154.25	$C_{10}H_{18}O$	0.28
19	1-methyl-4-(6-methylhept-5-en-2-yl)benzene	Sesquiterpene	16.63	202.34	$C_{15}H_{22}$	0.58
20	(*R*)-1-methyl-4-(6-methylhept-5-en-2-yl)cyclohexa-1,4-diene		16.93	204.36	$C_{15}H_{24}$	0.24
21	3,7,11-trimethyldodecan-1-ol		18.15	228.42	$C_{15}H_{32}O$	3.2
22	Rhodopin	Diterpene	42.79	554.0	$C_{40}H_{58}O$	0.29
Fatty acids and lipids						
23	tetradecan-2-yl 2-methoxyacetate	Ester of fatty acid	17.92	286.46	$C_{17}H_{34}O_3$	1.96
24	tetradecan-2-yl 2-methoxyacetate		17.92	286.46	$C_{17}H_{34}O_3$	1.96
25	tetradecanoic acid	Fatty acid	24.05	228.38	$C_{14}H_{28}O_2$	1.46
26	(Z)-7-methyltetradec-8-en-1-yl acetate	Ester of fatty acid	25.23	268.44	$C_{17}H_{32}O_2$	0.28
27	methyl palmitate		26.24	270.46	$C_{17}H_{34}O_2$	3.09
28	palmitic acid	Fatty acid	28.16	256.43	$C_{16}H_{32}O_2$	3.55
29	methyl (*E*)-octadec-11-enoate	Ester of fatty acid	29.47	296.50	$C_{19}H_{36}O_2$	4.42
30	methyl stearate		29.95	298.51	$C_{19}H_{38}O_2$	2.06
31	(9Z,12Z)-octadeca-9,12-dienoic acid	Fatty acid	31.08	280.45	$C_{18}H_{32}O_2$	0.57
32	oleic acid		31.61	282.47	$C_{18}H_{34}O_2$	14.96
33	(E)-octadec-13-enoic acid		32.22	282.47	$C_{18}H_{34}O_2$	0.38
34	methyl12-(2-octylcyclopropyl) dodecanoate		33.36	366.63	$C_{24}H_{46}O_2$	0.43
35	2-(tetradecyloxy)ethyl palmitate	Ester of fatty acid	34.11	496.86	$C_{32}H_{64}O_3$	0.32
36	2,3-dihydroxypropyl palmitate		36.04	330.51	$C_{19}H_{38}O_4$	0.79
37	methyl docosanoate		36.49	354.62	$C_{23}H_{46}O_2$	0.33
38	(Z)-icos-13-enoic acid	Fatty acid	37.36	310.52	$C_{20}H_{38}O_2$	0.90
39	(Z)-octadec-11-enoic acid		38.59	282.47	$C_{18}H_{34}O_2$	2.41
40	2,2,8,8-tetramethyl-3,7-dioxa-2,8-disilanonan-5-yl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate	Ester of fatty acid	42.36	496.88	$C_{27}H_{52}O_4Si_2$	0.99
Steroids						
41	Estra-1,3,5(10)-trien-17α-ol	Steroid	29.32	256.39	$C_{18}H_{24}O$	0.30
42	9,19-Cyclolanost-24-en-3-ol, acetate, (3α)-		41.10	468.77	$C_{32}H_{52}O_2$	0.83
43	9,19-Cyclolanost-24-en-3-ol, (3α)-		41.78	426.73	$C_{30}H_{50}O$	1.66
44	stigmast-5-en-3-ol, (3α,24S)-		43.97	414.72	$C_{29}H_{50}O$	1.84
45	Ethyl 3,7,12-trihydroxycholan-24-oate		45.00	436.63	$C_{26}H_{44}O_5$	1.17
46	Lup-20(29)-en-3-ol, (3α)-		47.72	426.73	$C_{30}H_{50}O$	3.57
Total						**100.00**

Table (5): Assessment of the Antibacterial activity of investigated plant extracts against pathogenic bacteria using different solvents. Inhibition zone diameter (mm) used to measure antimicrobial activity.

Bacterial pathogens	*P. floccosa*			*G. coronaria*		
	Solvent used					
	Water	Methanol	Petroleum ether	Water	Methanol	Petroleum ether
	Diameter of inhibition zone (mm)					
Bacillus cereus	-	-	-	12	12	-
B. subtilis	20	20	-	-	-	-
Enterobacter cloacae	-	-	-	-	-	-
Escherichia coli	-	-	-	-	-	-
Listeria monocytogenes	-	-	-	10	10	-
Pseudomonas aeruginosa	-	-	-	-	-	-
Salmonella typhi	-	-	-	-	-	-
Staphylococcus aureus	11	11	-	13	12	-
S. epidermidis	-	-	-	10	10	-

-, no inhibition zone detected.

and the ability to maintain the stability of the chemical structure (Herborne, 1973). The results showed that the phytochemical content of each plant varied according to the type of extract (either organic or aqueous). Water extracts of *P. floccosa* and *G. coronaria* showed higher phenols, flavonoids, tannins, alkaloids and saponins than other extract types. Therefore, for an effective extraction of phenolic compounds from different plant parts, higher polar solvents were optimum than less polar solvents (Galanakis *et al.*, 2013). Water and methanol extracts were better solvents than petroleum ether for extracting phenolic compounds owing to their good solubility and polarity (Galanakis *et al.*, 2013).

The IC50 of the extract is inversely linked to the richness of its antioxidant capacity (Hazra *et al.*, 2010; Thouri *et al.*, 2017). Concerning solvent types, the antioxidant activities of both plant species' extracts were as follows: water> methanol > petroleum ether. These results were in accordance to those of several studies which have evaluated the correlation between the antioxidant activity phenolic content in plants and found that the higher total phenol and flavonoid contents result in high DPPH scavenging activity (Ebrahimzadeh and Bahramian, 2009). Due to the large number of hydroxyl groups in their structure, flavonoids play a significant role as *in-vitro* antioxidants.

Forty volatile components were identified in *P. floccosa*, including 19 components related to oxygenated and non-oxygenated hydrocarbons, while 46 volatile components were recorded in *G. coronaria*, including 17 hydrocarbons, five terpenes, 18 fatty acids and lipids, and six steroids. According to El Mokni *et al.* (2019), qualitative and quantitative analyses of the essential oil of Tunisian *P. floccosa* revealed the presence of 59 organic volatiles, chiefly from sesquiterpene hydrocarbons, oxygenated sesquiterpenes, oxygenated hydrocarbons, monoterpenes, phenyl derivatives, and monoterpene and long-chain hydrocarbons. Out of the 36 *Phlomis* taxa examined for essential oils by Çalış and Başer (2021), 28 taxa have either one of these two sesquiterpene hydrocarbons alone or both of them together as significant components. The following significant sesquiterpenes are also present in *Phlomis* oils: caryophyllene oxide, (Z)-β-farnesene, spathulenol, α-cubebene, β-eudesmol, and α-cedrene. Limonene, linalool, and αpinene monoterpenes were detected as the most common monoterpenes in *Phlomis* taxa. Analyses of the essential oils separated from various *Phlomis* species from different countries have shown that the chief constituents of the oils are β-caryophyllene and germacrene-D (Li *et al.*, 2010).

Qualitative and quantitative analyses of Tunisian *G. coronaria* oil allowed the identification of forty components, representing 96.58% of the total sample. Volatile oil profiles of Jordanian *G. coronaria* aerial parts revealed more than 60 components, accounting for approximately 99.6% and 99.7% of the total oil constituents of the flower head and aerial except for flower heads, respectively (Twaha and Hudaib, 2010). The oil was featured by abundant levels of monoterpenes and moderate levels of sesquiterpenes. Flamini *et al.* (2003); and (Senatore *et al.*, 2004) in Europe examined the essential oil content of *G. coronaria* and found varying rates of camphor (45.8-22.1%) and cis-chrysanthenyl acetate (5.4 - 19.9%). The discrepancy in essential oil compounds can be attributed to the organic nutrients in the soil, as shown by Alvarez-Castellanos and Pascual-Villalobos (2003). However, the sampling sites located within the same bioclimatic conditions.

The primary constituents of the essential oil of G. *coronaria*, According to Alvarez-Castellanos *et al*. (2001), are camphor α-pinene, lyratyl-acetate, and β-pinene. This composition pattern is unusual among essential oils of previously reported *Chrysanthemum* species. Therefore, we can hypoth-esize that the chemical composition and quantity of the essential oil of *Glebionis* (*Chrysanthemum* sp.) depend on the

species, plant part, geographic origin, environ-ment, extraction and analytical procedures. Furthermore, the presence of numerous chemotypes, reflected in the variability in the chemical composition of *G. coronaria* essential oils from discrete areas can be advised too. A water extract of *P. floccosa* showed an inhibitory effect against *B. subtilis* and *S. aureus*. While, water and methanol extracts of *G. coronaria* displayed an inhibitory effect against *B. cereus*, *L. monocytogenes*, *S. aureus* and *S. epidermidis*. Different biological activities of *G. coronaria* are associated with the heterogeneity of its chemical composition (Senatore *et al.*, 2004; Zheng *et al.*, 2004). According to a study by Wijaya *et al.* (2020), the methanolic extract of *G. coronaria* had an antibacterial impact on gram-positive bacteria but not on gram-negative bacteria like *E. coli* or *P. aeruginosa*. These effects were said to be closely related to the plant's phenol and essential oil levels (Wijaya *et al.*, 2020).

CONCLUSION

This study evaluated the phytochemical content, antioxidant properties, and antimicrobial activities of three types of extracts (water, methanol, and petroleum ether) from the aerial parts of *Phlomis floccosa* and *Glebionis coronaria* plants. A comparison between the two species revealed that aqueous extracts had the highest phytochemical content and demonstrated greater radical scavenging activity compared to other extract-types. Additionally, the water extracts of both *Phlomis floccosa* and *Glebionis coronaria* plants exhibited potential antibacterial activity against certain bacterial pathogens. Meanwhile, the chemical composition of essential oils revealed 40 and 46 volatile components in *P. floccosa* and *G. coronaria* respectively, consistent with previous studies but with minor variations that could be attributed to environmental factors. Further investigation reveals significant potential for future use of these two plants as sources of natural products or food preservatives due to their noteworthy antioxidant and antibacterial activities.

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المحتوى الفيتوكيميائى ومضادات الأكسدة و الزيوت الأساسية والقدرة المضادة للبكتيريا لنباتى *Phlomis floccosa* و *Glebionis coronaria*

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الملخص العربـــــي

تتميز النباتات البرية الطبية بخصائص علاجية ودوائية مميزه نتيجة إحتوائها على العديد من المواد الثانوية النشطة. فى هذه الدراسة تم تقدير المواد الفيتوكيميائية فى مستخلصات الماء والميثانول والايثير البترولى فى نباتي *Phlomis floccosa* و *Glebionis coronaria* وكلاهما موجود بكثرة فى مدينة مرسى مطروح . تم تقييم نشاط مضادات الاكسدة بإستخدم DPPH وتم أيضا تحديد الزيوت الأساسية فى كلا النباتين بطريقة الGC-MS. كما تم تقييم الفعالية كمضادات لتسعة عزلات من البكتيريا الممرضة. مقارنة بالمستخلصات الأخرى أظهرت المستخلصات المائية فى كلا النباتين تركيزات عالية من المواد الفينولية والفلافونيدات والتانينات والقلويدات والصابونين. كما أظهر أيضا المستخلص المائى نشاط عالى كمضادات أكسدة. فى نبات *P. floccosa* تم التعرف على 40 مركب متطايرا ينتمى للزيوت الأساسية بينما تم التعرف على 46 مركب متطاير فى نبات *G. coronaria*. أظهر المستخلص المائى لنبات *P. floccosa* تأثير مثبط لنمو بكتيريا *Bacillus subtilis and Staphylococcus aureus* بينما أظهرت مستخلصات الماء والميثانول لنبات *G. coronaria* تأثير مثبط لنمو *Bacillus cereus, Listeria monocytogenes, S. aureus and S. epidermidis*. تشير النتائج التي توصلنا إليها إلى أن هذه النباتات يمكن أن تكون مصادر للمنتجات الطبيعية ذات أنشطة مضادة للأكسدة والبكتيريا الممرضة.