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Original article

Investigation of the Effect of Two Different Solvents (Aqueous and Alcoholic) on Phytochemical Screening and Antimicrobial Activities for the *Globularia Repens* Plant

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A B S T R A C T
This study aimed to investigate and evaluate the antimicrobial potential of leaves and stems of the *Globularia repens* plant growing in the Al-Jabal Al-Akhdar region, Libya. Plant parts were extracted using two solvents (aqueous and methanol). Phytochemical screening and paper chromatography were conducted to identify major classes of secondary metabolites. The antimicrobial activity was tested against six bacterial species (*including Bacillus cereus, Streptococcus pneumoniae, Escherichia coli,* and *Pseudomonas aeruginosa*) and two fungal species (*Alternaria alternata* and *Penicillium sp.*) using the agar well diffusion method. Phytochemical screening revealed the presence of carbohydrates, glycosides, tannins, flavonoids, sterols, and saponins. The antimicrobial assays demonstrated that the methanol extracts exhibited significant inhibitory activity against some tested pathogens, while the aqueous extracts were less effective. The potency of the extracts also varied between the leaves and stems.

Introduction

The secondary metabolites are widely distributed in plants, recognized for their significant antioxidant, antimicrobial, and therapeutic properties [1]. The extraction and characterization of these compounds from plant tissues, such as leaves and stems, are critical for understanding their bioactivity and potential applications in food, pharmaceutical, and environmental sectors [2]. *Globularia* species, including G. repens, have attracted increasing scientific interest due to their rich phenolic profiles and traditional medicinal uses in the Mediterranean region [3]. Efficient extraction of effective compounds is essential for maximizing yield and preserving bioactivity. Conventional methods such as Soxhlet, maceration, and solvent extraction remain widely used, but recent advances favor green and ultrasound-assisted extraction for improved efficiency and sustainability. The choice of solvent, temperature, and extraction time significantly influences the recovery and stability of active compounds, with ethanol-water mixtures often providing optimal results. Optimization of these parameters is particularly important for structurally diverse natural compounds found in plant matrices [4]. Plant-derived effective compounds exhibit broad-spectrum antimicrobial activity, inhibiting the growth of pathogenic bacteria and fungi, and disrupting biofilm formation. These properties make them promising candidates for natural preservatives and therapeutic agents [5]. Extracts from *Globularia* species have shown significant antibacterial, antifungal, and antibiofilm effects, supporting their traditional use and potential for novel antimicrobial applications [6]. The unique environmental conditions of the Al–Gabal Al–Khder region may influence the phenolic composition and bioactivity of *G. repens*, underscoring the importance of region-specific studies. Investigating the solvent extraction and microbial applications of natural product compounds from G. repens in this region will contribute valuable insights into their chemotaxonomic diversity and biotechnological potential [7-8]. The use of plant extracts was carried place in numerous studies in Libya during the last twenty years [9-30], also many studies were carried out on any samples of plants [31-45], soils and waters [46-75], to evaluate the chemical contents [76 -90]. This study aims to investigate the effect of some solvents (water and alcohol) on the phytochemical screening and their anti-microbial activities on the *Globularia repens* plant collected from the Al-Jabal Al-Akhdar region.

Methods

Preparation of samples

The parts (leaves and stems) of *Globularia repens* were collected during the peak flowering season in spring from various locations in the Al-Jabal Al-Akhdar region (Green Mountain) of northeastern Libya. This region features a Mediterranean climate and an altitude range of 500 to 800 meters above sea level. Collection sites

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were selected for their minimal exposure to environmental pollutants and agricultural chemicals to ensure the purity of the phytochemical constituents. The plant specimens were authenticated at the Seliphium Herbarium, Botany Department, Faculty of Science, Omar Al-Mukhtar University. After collection, the plant material was carefully cleaned with distilled water to remove dust and epiphytes. It was then shade-dried at room temperature (25°C ± 2°C) with adequate ventilation for 15–20 days. The dried material was ground into a fine powder (particle size ≤ 0.5 mm) using a commercial grinder (MIZA TB200) and stored in airtight, light-protected containers to prevent degradation until further Use [10-12].

Extraction Protocol

Based on polarity requirements for active extraction, two solvents of differing polarity were chosen: methanol (polar) and distilled water. All solvents, of analytical grade (purity ≥ 99.5%), were sourced from the laboratories of Omar Al-Mukhtar University and used without further modification. This selection is supported by previous studies [9] confirming the efficacy of these solvents in extracting natural active compounds across a range of polarities. The samples were dried and ground, then the extraction was carried out by taking 10 grams of the plant powder with 100 ml of each solvent. The rotary evaporator was used at the temperature degrees for water (75 ^{0}C) and (55 ^{0}C) for alcohol. then the samples were cooled and filtered, and kept in glass bottles until use.

Phytochemical Screening

Qualitative Phytochemical Analysis

A preliminary qualitative phytochemical analysis was conducted on all extracts to detect the presence of major classes of bioactive compounds. The screening followed established standards and previous procedures [28], with minor modifications. The specific assay methods used for each phytochemical class are summarized in (Table 1)

Table 1. Methods for qualitative phytochemical screening

Phytochemical Class Detected	Test Method
Sterols and/or Triterpenes	Libermann-Burchard's test
Flavonoids	Alkaline reagent test
Alkaloids	Dragendorff's test
Tannins	Ferric chloride test
Carbohydrates and/or Glycosides	Molish's test
Cardiac Glycosides	Keller-Killiani test, Kedde's test
Anthraquinones	Bornträger's test, Modified-Bornträger's test
Saponins	Froth test

Paper Chromatography Analysis

All chemicals used were laboratory grade.
Materials for paper chromatographic studies:
A. Sheets of Wathmann filter paper No.1.
B. Chromatographic glass tanks.

Solvent systems

The solvents used in this study were selected as follows:
A. Acetic acid: Water (15:85 v/v).
B. Methylene chloride: Methanol: Water (60 :35: 5 v/v).
C. Benzene: Ethyl acetate: Acetic acid (12:4:0.5 v/v).

Spray reagents

Some of the spray solutions were used to detect some special compounds, including:
1-Ferric chloride for phenolic compounds:
1% Ferric chloride solution in ethanol yields a blue to green color [91].
2-Aluminum chloride for flavonoids:
1% Aluminum chloride solution in ethanol yields a yellow fluorescence color.
3-Potassium hydroxide for anthraquinone and phenolic compounds:
2% Potassium hydroxide solution, ethanol yields red or yellow color [92].

Microorganisms

The extracts were individually tested against pathogenic bacteria; the following bacteria were tested:

Bacterial strains

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The extracts were tested against the following pathogenic bacteria, which were obtained from the Microbiology Department of El-Bayda Hospital:

Gram-positive bacteria:
Three species of Gram-positive: *Staphylococcus aureus, Bacillus cereus,* and *Streptococcus pneumoniae.*

Gram-negative bacteria:
Three species of Gram-negative (*Escherichia coli, Pseudomonas aeruginosa,* and *Shigella vulgaris*).

Fungi
The fungal species selected for this study were (*Alternaria alternata* and *Penicillium sp*). These isolates were obtained from the Department of Microbiology, Faculty of Veterinary Medicine, Omar Al-Mokhtar University, El-Bayda, Libya.

Antimicrobial Activity and Minimum Inhibitory Concentration (MIC) Determination:
The antimicrobial activity of the plant extracts was assessed using the agar well diffusion method [93]. Briefly, Mueller-Hinton (MH) agar plates were inoculated with the bacterial strains, while Potato Dextrose Agar (PDA) plates were inoculated with the fungal strains. Following inoculation, wells were aseptically punched into the agar using a sterile cork borer. Each well was loaded with 30 µL of the plant extract at various concentrations (ranging from 0.8 to 0.00001 g/mL). The inoculated plates were subsequently incubated at 37°C for 24–48 hours for bacterial strains and at 28°C for 48–72 hours for fungal strains. The diameter of the inhibition zones was measured, and the results are presented as the mean of triplicate experiments. The Minimum Inhibitory Concentration (MIC) was determined for extracts that demonstrated inhibitory activity in the initial screening, following established protocols. The MIC assay was performed using a dilution method, where 30 µL of each serially diluted extract (across the concentration range of 0.8 to 0.00001 g/mL) was dispensed into the wells. The MIC was defined as the lowest concentration of the extract that completely inhibited visible microbial growth after the incubation period [94].

Results

Phytochemical screening studies:
Phytochemical screening of the leaves and stems of *Globularia repens* was performed for carbohydrates, glycosides, tannins, flavonoids, sterols, terpenes, saponins, anthraquinones, cardiac glycosides, and alkaloids. The results, summarized in (Table 2), indicate that the plant contains carbohydrates and/or glycosides, tannines, but lacks cardiac glycosides, saponins, steroids/triterpenoids, and alkaloids. The study further revealed that alcoholic extracts yielded higher concentrations of tannins, carbohydrates/glycosides, and flavonoids than aqueous extracts. Additionally, leaves were found to have a greater relative content of carbohydrates and tannins compared to stems. Both alcoholic and aqueous extracts were confirmed as a source of steroids and/or triterpenes. In contrast, anthraquinones were absent in aqueous extracts from the leaves and stems.

Table 2. Phytochemical screening of stems and leaves of the Globularia plant

Plant	**Globularia**			
Chemical Test	**Leaves**		**Stems**	
	Al	aq	Al	aq
Saponins	_	_	_	_
Tannines	+++	+	++	+
Carbhydrate and/or Glycosides	+	+	++	+
Alkaloids	_	_	\	_
Flavonoids	_	++	_	+
Anthraquinones	+	_	+	_
Steroids and/or Triterpenods	_	_	\	_
Cardiac Glycosides	_	_	_	_

(+) Present, Al (Alcoholic), Aq (Aqueous), (-) Absent, (/) Not done.

Paper chromatographic studies of the plants (leaves and stems):

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Subsequent paper chromatography analysis, informed by the initial phytochemical screening, utilized two solvent systems of different polarities to isolate compounds. The most significant finding was the detection of tannins and flavonoids. These compounds were identified based on their Rf values and the characteristic colors produced upon spraying with reagents like $FeCl_3$ and KOH (Table 3). Globularia plant: In the water extract of Globularia leaves, the flavonoids were found in both solvent extracts Benzene, Ethyl acetate, Acetic acid, and Hexane - Acetone mixture). For Globularia: Flavonoids in water extract of stems in solvents (Benzene - Ethyl Acetate- Acetic acid) were found (Table 3).

Table 3. Paper chromatographic investigation of alcohol and aqueous extract of Globularia leaves and stems

Solvent	Reagent	Globularia			
		Leaves		Stems	
		Al	Aq	Al	Aq
Benzene- Ethyl Acetate- Acetic acid (60:5:35)	FeCl3	_	green	green	_
	KOH	Yellow RF = 0.52	Yellow RF = 0.69	Yellow RF= 0. 34	Yellow RF= 0. 34
Hexan- Aceton (20:80)	FeCl3	green	green	green	green
	KOH	Yellow RF = 0.52	Yellow RF =0.52	Yellow RF = 0. 34	Yellow RF=0. 34

Chromatographic analysis indicated variation in the natural organic compound content of the studied plants. A bluish-green color suggested the presence of flavonoids and phenols, while the appearance of yellow spots after treatment with visualization reagents (KOH and $FeCl_3$) confirmed the presence of tannins.

Antimicrobial activity:
The antimicrobial activity of aqueous and methanolic extracts from the leaves and stems was evaluated against a panel of bacterial and fungal species. The results are presented in (Tables 4-9).

Gram-positive bacteria:
Bacillus cereus:
By applying different concentrations of the studied plant extracts against Bacillus cereus bacteria, the results in the table(4) showed that the inhibition zone and MIC in the alcoholic extract of *Globularia repens* leaves were recorded at 0.4 g/ml, while the inhibition zone for the alcoholic extract of *Globularia repens* stems was 0.8 g/ml. The results of aqueous extracts of *Globularia repens* Leaves showed that the inhibition zone was 0.8g/ml. As for the aqueous extract of *Globularia repens* stems, the inhibition zone was at 0.4g/ml.

Table 4. Effect of methanol and water Gloubira extracts against Bacillus cereus.

Concentration	*Gloubira Extracts*			
	Methanol		Water	
	Leaves	Stems	Leaves	Stems
0.1g/ml	1.5	2	2	1.5
0.2/ml	1	1.5	1.5	1
0.4g/ml	1	1	1	1
0.8 g/ml	N.A	1	1	N.A
0.01 g/ml	N.A	N.A	N.A	N.A
0.001g/ml	N.A	N.A	N.A	N.A
g/ml 0.0001	N.A	N.A	N.A	N.A
0.00001 g /ml	N.A	N.A	N.A	N.A

N.A.: Non-active

Streptococcus pneumonia:
(Table 5) showed the effect of different concentrations of the studied plant extracts against *Streptococcus pneumonia*. The results showed that the inhibition zone and MIC in the alcoholic extract of *Globularia repens* leaves were recorded at 0.2 g/ml, while the inhibition zone for the alcoholic extract of *Globularia repens* stems was 0.4 g/ml. For all aqueous extracts, the inhibition zone was recorded at 0.8g/ml.

Table 5. Antimicrobial activities of different concentrations of Globularia extracts against Streptococcus pneumoniae

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Concentration	*Globularia* Extracts			
	Methanol		Water	
	Leaves	Stems	Leaves	Stems
0.1g/ml	1.5	2	1.5	1.5
0.2/ml	1	1.5	1.5	1
0.4g/ml	N.A	1	1.5	1
0.8 g/ml	N.A	N.A	1	0.5
0.01 g/ml	N.A	N.A	N.A	N.A
0.001g/ml	N.A	N.A	N.A	N.A
g/ml 0.0001	N.A	N.A	N.A	N.A
0.00001 g /ml	N.A	N.A	N.A	N.A

Staphylococcus aureus

(Table 6) showed the effect of different concentrations of the studied plant extracts against Staphylococcus *aureus*. The results showed that the inhibition zone and MIC in all extracts were recorded at 0.8 g/ml, except for Globularia repens stems, where the inhibition zone was recorded at 0.2g/ml.

Table 6. Effect of methanol and water Gloubira extracts against Staphylococcus aureus.

Concentration	*Gloubira Extracts*			
	Methanol		Water	
	Leaves	Stems	Leaves	Stems
0.1g/ml	2	2	2	2
0.2/ml	1.5	1.5	1.5	1
0.4g/ml	1	1.5	1	NA
0.8 g/ml	1	1	1	NA
0.01 g/ml	N.A	N.A	N.A	N.A
0.001g/ml	N.A	N.A	N.A	N.A
g/ml 0.0001	N.A	N.A	N.A	N.A
0.00001 g /ml	N.A	N.A	N.A	N.A

Gram-negative bacteria

Shigla vulgaris:

Table 7 showed the effect of different concentrations of the studied plant extracts against *Staphylococcus aure*us. Where low concentrations (0.01 – 0.0001 g/ml) and high concentrations (0.1- 0.8 g/ml) of the extracts were used. The results showed that the inhibition zone and MIC in the alcoholic extract of Globularia repens leaves were recorded at 0.4 g/ml, while the inhibition zone for the alcoholic extract of Globularia repens stems was 0.8 g/ml. The results of aqueous extracts of Globularia repens Leaves showed that the inhibition zone was 0.8g/ml. As for the aqueous extract of Globularia repens stems, the inhibition zone was at 0.4g/ml.

Table 7. Effect of methanol extract of Globularia globularia leaves for high and low concentrations against Shigella vulgaris

Concentration	*Gloubira Extracts*			
	Methanol		Water	
	Leaves	Stems	Leaves	Stems
0.1g/ml	2	2	2	1
0.2/ml	1	2	1.5	1
0.4g/ml	1.5	1.5	1	1
0.8 g/ml	NA	1	1	N.A
0.01 g/ml	N.A	N.A	N.A	N.A
0.001g/ml	N.A	N.A	N.A	N.A
g/ml 0.0001	N.A	N.A	N.A	N.A
0.00001 g /ml	N.A	N.A	N.A	N.A

Escherichia coli

(Table 8) recorded the effect of different concentrations of the studied plant extracts against *Escherichia coli*. The results showed that the inhibition zone and MIC in all *alcohol* extracts *of Globularia* were recorded at 0.8g/ml. The results aqueous extract of *Globularia repens* leaves showed that the inhibition zone was 0.4g/ml. As for the aqueous extract of *Globularia repens* stems, the inhibition zone was at 0.2g/ml.

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Table 8. Antimicrobial activities of different concentrations of the Globularia extracts against Escherichia coli

Concentrations	*Globularia* Extracts			
	Methanol		Water	
	Leaves	Stems	Leaves	Stems
0.1g/ml	1.5	2	1.5	1.5
0.2/ml	1.4	1.5	1	1
0.4g/ml	1	2	1	N.A
0.8 g/ml	1	1.5	N.A	N.A
0.01 g/ml	N.A	N.A	N.A	N.A
0.001g/ml	N.A	N.A	N.A	N.A
g/ml 0.0001	N.A	N.A	N.A	N.A
0.00001 g /ml	N.A	N.A	N.A	N.A

Pseudomonas aeruginosa

(Table 9) illustrated the effect of different concentrations of the studied plant extracts against Pseudomonas aeruginosa. The results showed that the inhibition zone and MIC in the alcoholic extract of Globularia repens leaves were recorded at 0.2 g/ml, while the inhibition zone for the alcoholic extract of Globularia repens stems was 0.8 g/ml. The results of aqueous extracts of Globularia repens Leaves showed that the inhibition zone was 0.4g/ml. As for the aqueous extract of Globularia repens stems, the inhibition zone was at 0.2g/ml

Table 9. Antimicrobial activities of different concentrations of Globularia extracts against Pseudomonas aeruginosa

Concentration	*Globularia Extracts*			
	Methanol		Water	
	Leaves	Stems	Leaves	Stems
0.1g/ml	2	2	1.5	1.5
0.2/ml	1	1.5	1	1
0.4g/ml	N.A	1.5	1.5	N.A
0.8 g/ml	N.A	1	NA	N.A
0.01 g/ml	N.A	N.A	N.A	N.A
0.001g/ml	N.A	N.A	N.A	N.A
g/ml 0.0001	N.A	N.A	N.A	N.A
0.00001 g /ml	N.A	N.A	N.A	N.A

Antifungal activity

***Pensilienium*:**

(Table 10) recorded the effect of different concentrations of the studied plant extract against *Pensilienium*. The results showed that the inhibition zone and MIC at 0.001g/ml for Globularia Leaves of alcohol extract, The results of aqueous extracts of Globularia repens Leaves showed that the inhibition zone was 0.01 g/ml g/ml. As for the aqueous extract of Globularia repens stems, the inhibition zone was at 0.4g/ml.

Table 10. Antifungal activities of different concentrations of Globularia extract against Pensilienium

Concentration	*Globularia* Extracts			
	Methanol		Water	
	Leaves	Stems	Leaves	Stems
0.1g/ml	2	NA	2	2
0.2/ml	1.5	NA	2	2
0.4g/ml	2.5	NA	1.5	2
0.8 g/ml	1.5	NA	1	NA
0.01 g/ml	1.5	N.A	1	N.A
0.001g/ml	1	N.A	N.A	N.A
g/ml 0.0001	N.A	N.A	N.A	N.A
0.00001 g /ml	N.A	N.A	N.A	N.A

Alternaria alternate:

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(Table 11) showed different concentrations of the studied plant extract against *Alternaria alternata*. he results showed that the inhibition zone and MIC at 0.2g/ml for Globularia Leaves of alcohol extract. The aqueous extracts of Globularia repens Leaves showed that the inhibition zone was 0.8 g/ml g/ml. As for the aqueous extract of Globularia repens stems, the inhibition zone was at 0.4g/ml.

Table 11. Antifungal activities of different concentrations of the Gloubirla extract against Alternaria alternata

Concentration	*Gloubirla* Extracts			
	Methanol		Water	
	Leaves	Stems	Leaves	Stems
0.1g/ml	1	NA	2	3
0.2g/ml	1	NA	1	2
0.4g/ml	NA	NA	1	1.5
0.8 g/ml	NA	N.A	0.5	N.A
0.01 g/ml	N.A	N.A	N.A	N.A
0.001g/ml	N.A	N.A	N.A	N.A
g/ml 0.0001	N.A	N.A	N.A	N.A
0.00001 g /ml	N.A	N.A	N.A	N.A

Discussion

The variation in detected compounds between extracts stems directly from the differing polarities of the solvents (water and alcohol) and the inherent chemical structures of the phytochemicals. These factors collectively determine solubility, explaining why certain compounds are extracted from specific plant tissues but not others, even if present in the raw material. Analysis of the growth inhibition zones revealed that the methanol extract was the most effective, exhibiting the highest activity against all selected bacterial strains. The maximum zones of inhibition measured 1-2 mm for Staphylococcus aureus and 0.5-2 mm for Streptococcus pneumoniae. A clear dose-dependent response was observed, with all extracts exhibiting more potent inhibition at higher concentrations (0.1 – 0.8 g/mL) compared to lower concentrations (0.00001 – 0.01 g/mL). In contrast, the aqueous extract showed negligible activity and was only effective against Streptococcus pneumoniae and Escherichia coli at the highest concentrations tested. In general, both Gram-positive and Gram-negative bacteria demonstrated increased susceptibility to the extracts at higher concentrations. The variation in antimicrobial efficacy of the different solvent extracts can be attributed to the polarity of the solvents, which determines the extraction efficiency of specific bioactive compounds with antibacterial properties [95].

The differential susceptibility of the tested bacterial strains to the plant extracts can be explained by the fundamental structural differences in their cell envelopes, particularly the thick peptidoglycan layer in Gram-positive bacteria compared to the complex outer membrane of Gram-negatives [96]. The various tested extracts reduced the colony diameters of the fungal strains. The aqueous and methanolic extracts of the Globularia plant exhibited antifungal activity against all tested strains. This activity was more pronounced at higher extract concentrations (0.1 –0.8 g/mL) compared to lower concentrations (0.00001 –0.01 g/mL). inhibition zones ranging from 0.5 to 2.5 mm. Although the precise mechanism of action of these plant constituents is not yet fully understood, it is clear that the effectiveness of the extracts largely depends on the type of solvent used. His observation clearly indicates the presence of non-polar residues in the extracts that possess significant antifungal abilities.

The superior efficacy of the methanol extract over the aqueous one aligns with recent findings that attribute this difference to the ability of organic solvents to extract a wider spectrum of antimicrobial compounds. This disparity can be attributed to the distinct chemical composition of the extracts. The activity of non-polar compounds is often linked to mechanisms such as disrupting fungal cell membrane integrity [28]. Furthermore, the percent inhibition of the plant extracts on pathogen growth varied with increasing concentrations, a common dose-response relationship documented in contemporary studies [97-98]. Therefore, using higher concentrations or alternative extraction methods could be investigated to achieve a more potent effect against all strains.

Conclusion

The findings demonstrate that Libyan *Globularia repens* is a rich source of bioactive compounds. The antimicrobial efficacy is significantly influenced by the extraction solvent, with methanol being more effective than water. These results support the potential use of these plants, particularly their methanol extracts, as natural antimicrobial agents.

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Conflict
No conflict of the results given in this study with other scientific works

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