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Phytochemical Study and Potential of Antibacterial Performance of *Dittrichia Viscosa* Subsp. Viscosa

Salawa I.A. Eltawaty[1*], Tawfeek Altawaty[2], Mohammed B. Suliman[3], Heba Khalifa[4], Farag Shouaib[4]

[1] Department of Biomedical Science, Faculty of Pharmacy, Omar Al-Mukhtar University, Al-Bayda, Libya

[2]Department of Molecular Diagnostics, Faculty of Biomedical Sciences, University of Benghazi, Benghazi, Libya

[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Omar Al-Mukhtar University, Al-Bayda, Libya

[4]Department of Botany, Faculty of Science, Omar Al-Mukhtar University, Al-Bayda, Libya

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KEYWORDS
Dittrichia viscosa, GC-MS Analysis, Antibacterial performance.

ABSTRACT:
Dittrichia viscosa (*D. viscosa*) is a native distributed species around Albayda City, northeast of Libya. Although it has been previously studied, but with the different ecosystems in the study region, this study is the first. Therefore, this study aimed to determine the phytochemical composition and antibacterial effects of methanol extracts of root, stem, and leaf extracts of *D.viscosa* native to Albayda City, Libya. Methanol, Soxhlet, and rotary evaporator were used for solubility, extraction, and evaporation. The agar well and agar disc diffusion methods were used to evaluate the antibacterial activities of extracts and antibiotic references, respectively. The results showed broad antibacterial activities against MDR bacteria with inhibition zones ranging from (15.5+0.2 to 29±0.2), the highest was 29mm from the leaf and 27.5±0.1 from root extracts against *P. aeruginosa* followed by 24±0.1 from root extract against *Acinetobacter baumannii*. The investigation of phytochemicals proved that the plant consists of the most common bioactive compounds previously confirmed for their antibacterial potential. Ethyl.alpha.-d-glucopyranoside (14.88%), 1-Deoxy-d-mannitol (14.45%), Quinic acid (13.4%), beta.-D-glucopyranoside, methyl (12.95%), 9,12-Octadecadienoic acid (Z,Z)- (4.67%), Neotigogenin (4.58%), and gamma – Sitosterol (2.73%) were the highest major compound. This study concludes that *Dittrichia viscosa* plant could be considered a natural source of compounds that help for promoting patients health by fighting harmful bacterial pathogens such as Methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baummannii*.

Introduction:

Medicinal plants have long been a cornerstone of traditional medicine across cultures worldwide. Recently, the scientific community has rediscovered their therapeutic potential, particularly in the treatment of bacterial infections. As antibiotic resistance continues to rise, the need for alternative or adjunct therapies has never been more urgent. Medicinal plants offer a promising solution due to their broad spectrum of bioactive compounds which have demonstrated antimicrobial properties in vitro and in vivo. Medicinal plants offer to exert their antibacterial action via various mechanisms. Those consist of disruption of the bacterial cell wall, inhibition of protein synthesis, and interference with bacterial DNA replication. For instance, the antimicrobial interest of Tea tree oil (*Melaleuca alternifolia*) is attributed to its ability to disrupt the bacterial cellular membrane, main to leakage of mobile contents and eventual mobile dying (Carson et al., 2002). Further compounds discovered in Turmeric (*Curcuma longa*), inclusive of curcumin, have been shown to inhibit bacterial virulence factors and biofilm formation, which might be crucial for bacterial survival and resistance to continual infections (Bansal et al., 2013).

A sizable number of plants were proven to show antibacterial strategy against a huge range of pathogens. For instance, ***Dittrichia viscosa*** is well known for its

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antimicrobial performance against Gram-positive and Gram-negative bacteria. *Dittrichia viscosa*, usually known as the golden samphire or fake goldenrod, is a plant species extensively recognized for its wide traditional medicinal usage, such as its antibacterial performance. The plant's leaves, stems and roots have been reported to exhibit a promising antibacterial activity toward pathogenic microorganisms. The plant consists of bioactive compounds including flavonoids, terpenoids, phenolic compounds, and essential oil, which are believed to contribute to its antimicrobial capability. Especially, the leaves have been reported to have an amazing impact against Gram-positive bacteria such as Methicillin-resistant *Staphylococcus aureus*, which is tough to deal with because of its resistance to B-lactam antibiotics. This pathogen (MRSA) is notorious for its resistance to many antibiotics, along with Methicillin (Gomes et al., 2013). Previous studies concluded that the leaves, stems, and roots of *Dittrichia viscosa* are effective against the Gram-positive bacterium; *Bacillus subtilis*, and also against the Gram-negative bacterial pathogens; *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, which are usually associated with hospital-acquired infection nad due to their multi-drug resistance are considered as critical pathogens in hospital settings (Müller et al., 2017; Costa et al., 2019; Zahou et al., 2020, Vuko et al., 2012; Mssillou et al., 2022). The *Pseudomonas aeruginosa* is described as a versatile and opportunistic pathogen is proof against an extensive range of commonly used antibiotics. The antibacterial capability of *Dittrichia viscosa* in opposition to *Pseudomonas aeruginosa* has been validated in a few research, suggesting that plant extracts might be a unique method for handling infections caused by this pathogen (Zouaghi et al., 2021; Mird et al., 2022). To harness the overall capacity of medicinal vegetation in fighting bacterial infections, there is a want for broader research relating the traditional use with cutting-edge pharmacological techniques. In this context, this study aimed to phytochemically analyze the contents of leaves, stems, and roots of *Dittrchia viscosa* and test its antibacterial efficacy.

Materials and Methods

Collection and Authentication of Plant Materials

The roots, stems, and leaves of *D. viscosa* were collected in September 2023 in Al-Jabal Al-Akhdar region (Northeast of Libya). The plant was identified by the herbalists at the Seliphum herbarium, Botany Department, Faculty of Science, Omar Al-Mukhtar University, Al-Bayada, Libya. All the samples were washed and rinsed with distilled water. The samples were dried, crushed using a mortar and pestle, and finally reduced to fine particles using a mechanical blender (Bajaj GX10, India). They were then stored in airtight closed bottles before being used for analysis.

Microbial Strains

The antibacterial property of *D. viscosa* extracts was tested against the following microorganisms: *Methicillin-resistant Staphylococcus aureus* (MRSA), *Bacillus subtilis* (NCTC 8236), *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* (ATCC 27853). All strains were provided by the Laboratory of Biomedical Science at the Faculty of Pharmacy, Omar Al-Mukhtar University, Al-Bayda, Libya.

Chemicals for Antibacterial Activity

Four commercial antibiotic discs, representing different antibiotic classes with unique mechanisms of action, were used as references in this study. The antibiotics included were Augmentin (30 µg), Cefoxitin (30 µg), Cefotaxime (30 µg), and Meropenem (10 µg). All antibiotic references were purchased from local private companies.

Preparation of Methanol Crude Extracts

Exactly, 20 g of each shade-dried and powdered plant part (roots, stems, and leaves) were extracted using a Soxhlet apparatus for six hours with methanol (250 mL). The methanol was then evaporated using a rotary evaporator, further dried in the open air, and stored in dark bottles at 4°C until analysis or evaluation for antibacterial activity.

Preliminary Phytochemical Analysis

Phytochemical screening of *D.viscosa* was done with the roots, stems, and leaf extracts by following the standard procedures (Edeoga et al., 2005; Yadav et al., 2011). All crude extracts were examined for the presence ofsteroids,

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flavonoids, alkaloids, tannins, terpenoids, anthraquinones, and saponins.

GC-MS Analysis

The compounds present in root and leaf extractsof *D.viscosa* were determined using gas chromatography-mass spectrometry (GC-MS). For the GC-MS method, an Agilent Technologies 7890A GC System coupled with an Agilent Technologies 5975C mass spectrometry detector GC-MS system, equipped with a DB-1 capillary column (30 m x 0.25 mm ID, film thickness 0.25 µm) was applied. The temperature started at 60ºC for two min, increased at a rate of 5ºC/min, and was finally maintained at 200ºC for four min. The total analysis time was 34 min, and the flow rate was 1.5 mL/min with helium as the carrier gas. The injector temperature was maintained at 240ºC, and the mass range scanned was 3-500 *m/z* (Mohammed et al. 2015). The compounds in the GC-MS analysis were identified based on a comparison of the retention time and mass spectra with the references present in the NIST mass spectral library.

***In vitro* Antibacterial Activity of *D. viscosa* Extracts**

The *in vitro* antibacterial activity of the methanolic extract from the roots, stems, and leaf of *D. graveolens* was examined. The antibacterial activity of these extracts was evaluated using the agar well diffusion method as described by Magaldi et al. (2004) with minor modifications. For all bacterial strains, overnight cultures grown in broth were adjusted to an inoculum size of 1.5×10^8 colony-forming units (CFU)/mL using a 0.5 McFarland standard and inoculated onto agar plates. The plates were allowed to sit for five min to allow the diffusion of the extracts, after which 50 µl of the extract being tested was added to each well (7 mm diameter holes were cut into the agar gel). The plates were then incubated at 37℃ for 24 h, and the diameters (mm) of the inhibition zones were measured in millimetres. Triplicate wells were used for each extract against each of the tested organisms, and the mean values were calculated.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using the serial dilution method described by Andrews (2001) with slight modifications. Initially, serial dilutions were prepared for each extract in decreasing concentrations in the following order: 200, 100, 50, 25, 12.5, 6.25, and 3.125 mg/mL. In sterile, covered glass bottles, 5 ml of melted double-strength Mueller-Hinton agar (MHA) cooled to 45°C were mixed with 5 mL of each dilution of the tested plant extract to achieve final serial dilutions of 100, 50, 25, 12.5, 6.25, and 3.125 mg/mL for each extract. The mixture was poured into sterile small petri dishes, allowed to solidify, and then the bottom of each plate was marked into segments. A standard loop (100 µL) of each tested bacterial fresh suspension adjusted with McFarland 0.5 solution was spotted onto the surface of each segment of the marked MHA. The inoculums were allowed to be absorbed into the agar before being incubated at 37°C for 18 h. After the incubation period, the lowest concentration in mg/mL of the plant extract that completely inhibits the growth of the bacterium is considered the MIC.

Statistical Analysis

All the data were expressed as mean ± SD (standard deviation) and all statistical analyses were performed using the SPSS statistical software package (SPSS Inc., Chicago, IL, USA).

Results of Phytochemical Analysis

The traditional phytochemical analysis used in this study has proved the presence of the flavonoid group in the extracts of all plant parts. At the same time, alkaloids and tannins appeared in the extracts of roots and stems. The terpenoids in this study appeared only in the stem extract. No presence was shown for other investigated Phyto-groups; saponins, anthraquinones, and steroids (Table 4).

Table 4: Result of traditional Phytochemical analysis of *Dittrichia viscosas* extracts

	Alkaloids	Saponins	Terpenoids	Flavonoids	Anthraquinones	Steroids	Tannins
Root	Present	Absent	Absent	Present	Absent	Absent	Present
Stem	Present	Absent	Present	Present	Absent	Absent	Present

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Leaves	Absent	Absent	Absent	Present	Absent	Absent	Absent

The retention time and quantity of compound constituents detected in the roots extract of *Dittrichia viscosa* by the use of GC-MS analysis are expressed in (Table 2). The results proved the presence of 25 compounds in the root extract. The seven compounds of highest presence percentage were Ethyl.alpha.-d-glucopyranoside (14.88%), 1-Deoxy-d-mannitol (14.45%), Quinic acid (13.4%), beta.-D-glucopyranoside, methyl (12.95%), 9,12-Octadecadienoic acid (Z,Z)- (4.67%), Neotigogenin (4.58%), and gamma – Sitosterol (2.73%) as shown in Table 5.

Table 5: Result of GC-MS analysis of methanol root extract of *Dittrichia viscosa*

Peak Report TIC

Peak#	Name	R.Time	Area	Area%
1	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	6.157	416673	1.30
2	1-Penten-3-ol, 2-methyl-	6.222	301741	0.94
3	1-Tridecene	6.757	456256	1.42
4	2-Cyclohexyl-3-isopropyl-pent-4-en-2-ol	7.221	561528	1.75
5	1,2,3-Propanetriol, 1-acetate	7.465	308026	0.96
6	1-Octanol, 2-nitro-	7.902	842777	2.63
7	1-Pentadecene	9.465	812211	2.53
8	Glutaric acid, di(2-nitrobenzyl) ester	10.137	463440	1.44
9	1-Heptadecene	11.939	839741	2.62
10	Quinic acid	12.135	4300062	13.40
11	.beta.-D-Glucopyranoside, methyl	12.313	4157143	12.95
12	Ethyl .alpha.-d-glucopyranoside	12.409	4774798	14.88
13	1-Deoxy-d-mannitol	12.608	4638732	14.45
14	Ethyl .alpha.-d-glucopyranoside	12.700	347771	1.08
15	2-[4-(2-Hydroxy-phenyl)-thiazol-2-yl]-5-phenyl-penta-2,4-dien	14.128	379681	1.18
16	1-Nonadecene	14.174	534456	1.67
17	n-Hexadecanoic acid	15.881	1342232	4.18
18	Hexadecanoic acid, ethyl ester	16.212	495052	1.54
19	9,12-Octadecadienoic acid (Z,Z)-	17.540	1499061	4.67
20	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	17.608	715044	2.23
21	Linoleic acid ethyl ester	17.809	431677	1.35
22	Bis(2-ethylhexyl) phthalate	21.071	437092	1.36
23	Spirostan-3-one, (5.alpha.,25R)-	27.194	689952	2.15
24	Neotigogenin	27.349	1470266	4.58
25	.gamma.-Sitosterol	27.456	875906	2.73
			32091318	100.00

Results of antibacterial assay:

This study analyzed and studied the antibacterial activity of the root, stem, and leaves of the *Dittrichia viscosa* (*D. viscosa*) plant. The results showed that the highest growth inhibition zone in (mm) revealed from **root extract** was (**27.5**±0.1) against *Pseudomonas aeruginosa* followed by (24±0.1) and (17.5±0.1) against *Acinetobacter baumannii*, and Methicillin-resistant *Staphylococcus aureus* (MRSA), respectively. No effect was shown from leaf extract on *Bacillus subtilis*. The highest growth inhibition zone in (mm) revealed from the **stem extract** was (**17.5**±0.1) against *Pseudomonas aeruginosa* followed by (17±0.3), (14.5±0.1), and (15.5+0.2) against tested *Bacillus subtilis*, *Acinetobacter baumannii*, and MRSA, respectively. For the **leaf extract**, the highest growth inhibition zone was (**29**±0.2)

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Pseudomonas aeruginosa followed by (20±0.1), (and 9±0.1) against MRSA and *Acinetobacter baumannii*, respectively. No effect was shown from leaf extract on *Bacillus subtilis* (Table 1 & figure 1). When the minimum inhibitory concentration was determined the results showed that the lowest concentration was 6.125mg/ml from the root extract followed by 12.5mg/ml from the leaf extract against MRSA (Table 2). When the activity the antibiotic disc references is tested against the targeted isolates, the highest growth inhibition zone revealed was with Cefotaxime (20±0.1) against *Bacillus subtilis* followed by (19±0.1) and (15±0.1) against *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, respectively. Meropenem affected only *Bacillus subtilis* and *Pseudomonas aeruginosa* with near-close inhibition zones; (20±0.1) and (19±0.2), respectively (Table 3 & Figure 1).

Table 1: Antibacterial activity (inhibition zone) of different extracts of *D. viscosa*

Microorganisms	**Diameter of inhibition zones (mm)**		
	Roots	Stems	Leaves
Methicillin-resistant *S. aureus*	17.5±0.1	15.5+0.2	20±0.1
A. baumannii	24±0.1	14.5±0.1	9±0.1
B. subtilis	0±0.0	17±0.3	0±0.0
P. aeruginosa	**27.5**±0.1	**17.5**±0.1	**29**±0.2

All values are presented as mean ± standard deviation (SD).

The concentration of each extract was 100 mg/ml.

Table 2: The MIC value of different extracts of *D. vicosa*

Microorganisms	**MIC/IC_{50} (mg/ml)**		
	Roots	Stems	Leaves
Methicillin-resistant *S. aureus*	6.125	100	12.5
A. baumannii	100	100	ND
B. subtilis	ND	100	ND
P. aeruginosa	100	100	100

All values are presented as mean ± standard deviation (SD).

Table 3: Antibacterial activity (inhibition zone) of antibiotic Disc references

Microorganisms	Cefotaxime (30 µg)	Meropenem (10 µg)
Methicillin-resistant *S. aureus*	0±0.0	0±0.0
A. baumannii	15±0.1	0±0.0
B. subtilis	20±0.1	20±0.1
P. aeruginosa	19±0.1	19±0.2

All values are presented as mean ± standard deviation (SD).

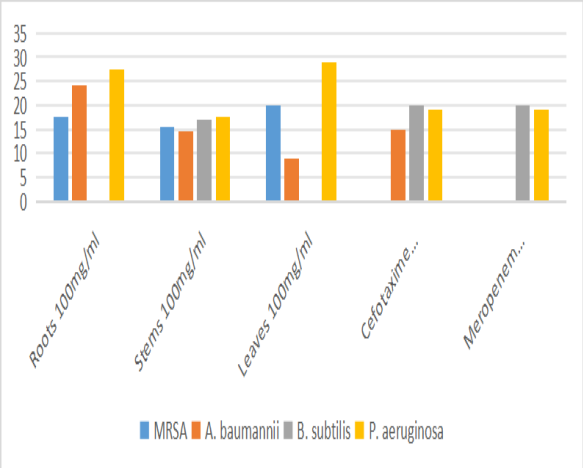

Figure (1): Antibacterial activity of different plant extracts and antibiotics

Discussion:

It has been reported that different parts of plants (root, stem, leaves, fruits) usually contain compounds as

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secondary metabolites proven for their antimicrobial potential. The most commonly reported having the strongest antimicrobial potential are flavonoids, tannins, alkaloids, and glycosides which suppress microbial growth either singly or synergistically with each other (El-Saadony et al., 2023).

Many medicinal wild-grow plants indigenous to the Al-Jabal Al-Akhdar region, northeast of Libya have antimicrobial properties and have not been studied yet. These plants are used traditionally for many ailments, for which they are thought to provide a great origin for deriving naturally potent antimicrobial agents.

Dittrichia viscosa is one of these plants targeted in this study to be phytochemically analyzed and investigated for its potential antibacterial activity against human pathogenic bacteria. The investigation proved that most of the different extracts of *Dittrichia viscosa* offered good growth inhibition performance against tested isolates. The stem extract appeared to be the broadest one, where it actively controlled all tested Gram-positive and Gram-negative clinical bacterial pathogens. Leaf extracts actively inhibited MRSA and *Pseudomonas aeruginosa*, but weakly inhibited the *Acinetobacter baumannii*, while the root and leaf extracts failed to control the tested *Bacillus subtilis*. This study is in agreement with Rhimi et al., (2017) who reported an inhibition zone (20 ± 0.0) which is close to what was revealed in this study against *Staphylococcus aureus*, with the note that they investigated the same part "leaves", and used the same solvent "methanol", but with different concentrations, where they tested (50mg/ml) instead of (100mg/ml) of the extract. Also, Grauso and his team agreed with this study and reported that the methanol extract of leaves of *D. viscosa* has good antibacterial activity against the *Bacillus subtilis* pathogen (Grauso et al., 2020).

Although this study proved the effectiveness of the leaf extract against both MRSA and *Pseudomonas aeruginosa* (20±0.1) and (29±0.2), respectively, Grauso's results on the same pathogens were inconsistent and proved that the leaf extract was ineffective against these two pathogens. Also, Grauso's results showed a moderate inhibition zone (10.3 ± 0.6) against *Bacillus subtilis* while in this study no effect was shown against this bacterium (Grauso et al., 2020). This inconsistency might be due to the different areas, seasons of plant collection, method of extraction, and also different solvent concentrations used. While this study targeted plant leaves collected from Libya in August (2023) and absolute methanol was used for extraction with the use of Soxhlet apparatus, Ali's study targeted leaves collected from Morocco in January (2018), and 80% methanol was used for extraction with maceration process. Many factors affect on type and concentration of a plant's bioactive compounds such as the geographic position of the plant, the harvesting season, the solvent used, and the technique used for extraction (Ghnaya et al., 2013; Abdulla-Eltawaty et al., 2012).

Where Methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baummannii* are on the top of the most worldwide problematic pathogens that threats patients' lives, the **root** extract showed a promising source for phytochemical compounds that can suppress these pathogens, since this extract showed inhibition zones higher than that revealed from the tested third-generation cephalosporin "Cefotaxime 30 µg" and the carbapenem "Meropenem 10µg". The phytochemical analysis interprets this promising result when the flavonoids are found present in all plant parts, in addition to the presence of Alkaloids, and tannin in the root and stem. Also, the presence of Quinic acid, Ethyl.alpha.-d-glucopyranoside, and gamma – Sitosterol as constituents to this plant stand behind the shown antibacterial activity where they were previously confirmed for their good antibacterial performance.

Conclusion

This study concludes that all parts of Dittrichia viscosa plant could be considered a natural source of compounds help for promoting health well-being by fighting the harmful bacterial pathogens such as Methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baummannii* .

Conflict of interest statement: We declare that we have no conflict of interest.

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