MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Phytochemical analysis and Biological Activity of *Verbena officinalis* L. in two habitats

Asmaa S. Nasr, Mohamed Abdelaal*, and Gamal Abd El-Fattah

Botany Department, Faculty of Science, Mansoura University, Mansoura 35516, Egypt

**** Correspondence to:*** *Email: mohamed_eco@mans.edu.eg, Tel: 01117370673*

Received:1/8/2023
Accepted: 20/8/2023

Abstract:The therapy of the medicinal plants has always been a part of our environment as they have several therapeutic virtues. These properties are generally attributed to secondary metabolites. Therefore, it is critical to highlight habitat conditions and quantify active phyocompounds to optimize their phytochmical constituents. Verbena officinalis is a perennial medicinal plant, known for its rich folkloric and traditional medicinal applications. The aim of this study was to test the biological activity and phytochemicals of V. officinalis from two different habitats, as well as to address the physicochemical features of soil where V. officinalis is growing. V. officinalis favored sandy soil with low salinity. In methanol extract of V. officinalis in coastal desert, phenols exhibited the highest level (54.32 mg gallic acid g^{-1} dry extract) followed by flavonoids, tannins, alkaloids and finally saponins (0.56 mg g^{-1} dry extract). V. officinalis in coastal desert showed a high antioxidant activity with IC50 value of 2.30 mg ml^{-1}. The extract exhibited better inhibition against gram +ve bacteria (S. aureus and B. subtilis), than that of gram -ve isolate (Escherichia coli). The extract of V. officinalis in Inland desert showed maximum antibacterial activity against Staphylococcus aureus and Bacillus subtilis with a diameter of 15.2 mm and 16 mm, respectively. Based on the study finding, V. officinalis contains potential antimicrobial components that may be of great use for the development of pharmaceutical industries as an alternative therapy against various bacterial diseases.

Keywords: medicinal plants, phytochemistry, antioxidant, antibacterial.

1.Introduction

The usage of medical plants, often known as medicinal herbs, dates back to ancient times. Huge amounts of chemical compounds are found in plants, which are used for defense against pests, diseases, fungi, and herbivorous mammals [1].

Numerous phytochemicals found in plants, such as tannins, alkaloids, flavonoids, and terpenoids, have been discovered in tests to have antibacterial properties [2].

Verbena officinalis, a plant from the Verbenaceae family, is commonly called pigeons' grass, herb of grace, or vervain. It is a perennial herb that stands upright and reaches a height of 25 to 100 cm. The leaves are toothed and lobed [3].

The most recent research on herb extracts from *Verbena* officinalis has revealed that, this plant's raw material is characterized by various beneficial biological properties, including antioxidant, antibacterial, antifungal and anti-inflammatory [4].

The aims of current study were to (1) address the physicochemical features of soil where *V. officinalis* is growing, 2) quantify the secondary metabolites in aerial parts of *V. officinalis* and 3) assess the antioxidant and antibacterial activities of *V. officinalis*.

2. Materials and methods

2.1. Plant collection

Th fresh aerial parts of *V. officinalis* were collected in March 2022 from two different habitats, coastal desert (along the Deltaic Mediterranean coast of Egypt) and inland desert (eastern Desert), where *V. officinalis* is growing. The plant samples were cleaned by running water then by distilled water, left for

air-dried at room temperature and finally powdered.

2.2. Soil- analysis

Three soil samples were collected at 50 cm depth and pooled as a composite. Physicochemical properties of soil were estimated according to Association of Official Analytical Chemists (AOAC) [5]. Na^{+}, K^{+}, Ca^{++} and Mg^{++} were determined.

2.3. Preparation of plant extract

Ten grams of air-dried *V. officinalis* powder was taken in 100 ml of 96% methanol in sterile conical flask, and placed on a rotary shaker for 24 hours, then filtered through Whitman filter paper and the supernatant was collected, then the filtrate was kept at 4°C till use.

2.4. Phytochemical analysis

Total phenols were estimated following the Folin-Ciocalteu [6]. The aluminum chloride assay was followed to determine flavonoids [7]. The vanillin-hydrochloride was used to weigh the tannin level [8]. Using ammonium hydroxide, the alkaloid was calculated [9]. Saponin was determined by the assay of [10].

2.5. Biological activity

2.5.1. Antioxidant activity by DPPH

Using ascorbic acid as a reference, the DPPH colorimetric method was used to assess the plant sample' antioxidant capacity and expressed as IC_{50} [10]. The IC50 value showed how many antioxidants were required to lower the concentration of DPPH solution by 50%.

2.5.2. Antibacterial activity

The methanol extract of *V. officinalis* was evaluated against three pathogenic bacterial strains (*Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*) using the agar well diffusion method [11]. After incubation, the diameter of inhibitory zone was measured (mm) and the average were obtained. Methanol was considered as a negative control.

3. Results

3.1. Soil properties

The physical and chemical properties of soil samples where *V. officinalis* is growing are displayed in **Table 1**. *V. officinalis* in coastal desert favored sandy soil with mean values of sand, silt and clay of 93.48 ,4.85 and 1.66%, respectively, while V. officinalis in inland desert attained the values of 79.57, 18.88 and 1.53 %, respectively.

The highest mean value (35.71%) of water holding capacity was recorded in *V. officinalis* of coastal desert, while the lowest mean value (32.58%) of soil porosity was recorded in V. officinalis in inland desert. Soil calcium carbonates ranged from 4.15% in coastal desert to 25.5% in Inland desert. Organic carbon varied from 0.15% in inland desert to 0.46 % in coastal desert. The highest mean values of total nitrogen and total dissolved phosphorus of 39.83 and 2.27 mg g^{-1} dry soil, respectively, were recorded in coastal deserts. The results of macroelements (Na^{+}, K^{+}, Ca^{++} and Mg^{++}) are displayed in **Table (1)**.

Table 1. Physical-chemical properties of soil samples collected from coastal and inland habitat of V. officinalis.

Soil factors	**Coastal**			**Inland**		
Soil factor	Min	max	mean±SE	min	max	mean±SE
Sand (%)	92.0	94.63	93.48±0.47	70.36	92.57	79.57±6.67
Silt (%)	3.37	5.9	4.85±0.68	7.25	26	18.88±6.22
Clay (%)	1.2	2.1	1.66±0.23	0.18	3.69	1.53±0.45
Porosity (%)	38.2	44.9	42.12±2.14	28.4	36.56	32.58±0.38
WHC (%)	34.12	36.95	35.71±0.93	23.83	31.12	27.67±1.79
$CaCO_3$ (%)	3.54	4.5	4.15±0.17	19.0	30.0	25.5±2.5
OC (%)	0.33	0.56	0.46±0.03	0.13	0.18	0.15±0.02
pH	7.54	8.27	7.85±0.13	8.3	8.8	8.6±0.25
EC	0.29	0.8	0.58±0.07	0.06	0.2	0.12±0.06
Cl^{-} (%)	0.11	0.95	0.58±0.07	0.03	0.04	0.03±0
SO_4^{--} (%)	0.32	0.55	0.40±0.07	0.03	0.78	0.36±0.02
HCO_3^{-} (%)	0.25	0.45	0. 33±0.03	0.18	0.34	0.25±0.04
TN	35.16	43.35	39.83±2.5	16.02	22.03	10.02±1.00
TDP	1.12	3.15	2.27±0.35	0.24	0.45	0.38±0.07
Na^{+} **(mg/100 g dry soil)**	31.28	115.94	78.05±7.33	0.07	0.19	0.11±0.01
K^{+}	8.55	25.44	16.27±3.91	0.14	0.25	0.19±1.01
Ca^{++}	11.06	34.75	23.24±3.05	1.98	2.64	2.21±0.31
Mg^{++}	7.85	16.09	11.09±2.05	1.63	4.69	3.19±0.98

WHC: water holding capacity, OC: organic carbon, EC: electric conductivity, TN: total nitrogen, TDP: total dissolved phosphorus, SE: standard error.

3.2. Phytochemical analysis of V. officinalis

The concentration of active phytocompounds in *V. officinalis* in two habitats is showed in **Table 2.** In methanol extract of *V. officinalis* in coastal desert, phenols exhibited the highest level (54.32 mg gallic acid g^{-1} dry extract) followed by flavonoids, tannins, alkaloids and finally saponins (0.56 mg g^{-1} dry extract).

Table (2): Phytochemical analysis (Mean±SE) of methanol extract of *V. officinalis*.

	Coastal	**Inland**
Phenols (mg gallic acid g^{-1})	54.32±1.53	48.54±0.90
Flavonoids (mg catechin g^{-1})	6.50±0.96	8.43±1.25
Tannins (mg tannic acid g^{-1})	2.87±0.54	3.50±0.80
Alkaloids (mg g^{-1} dry extract)	5.24±0.78	6.11±0.35
Saponins (mg g^{-1} dry extract)	0.56±0.01	0.88±0.02

3.3. Biological activity of V. officinalis

3.3.1. Antioxidant activity

The DPPH scavenging activity of methanol extract of *V. officinalis* is displayed in **Table (3)**. As compared with the natural antioxidant ascorbic acid (IC_{50}= 0.02 mg ml^{-1}), *V. officinalis* in coastal desert showed a high antioxidant activity with IC50 value of 2.30 mg ml^{-1}.

Table (3). Antioxidant activity (IC_{50}) and antibacterial activity of methanol extract of *V. officinalis* in two habitats.

IC50 (mg ml^{-1})			
Coastal Inland		**2.30 2.10**	
Antibacterial potential			
V.officinalis	Escherichiacoli	Bacillus subtilis	Staphylococcus aureus
	Inhibition zone (mm)		
Coastal	-	11	14
Inland	-	16	15.2
Methanol	-	-	-
DMSO	-	-	-

3.3.2. Antibacterial potential of V. officinalis

The methanol extract of *V. officinalis* showed comparable inhibitory effects against the tested bacterial strains **(Table 3)**. The extract exhibited better inhibition against gram +ve bacteria (*S. aureus* and *Bacillus subtilis*) than that of gram -ve isolate (*E. coli*). The extract of *V. officinalis* in Inland desert showed maximum antibacterial activity against *S. aureus* and *B. subtilis* with diameter of 15.2 mm and 16 mm, respectively.

4. Discussion

The soil characteristics of the native habitats where plants naturally grow must be addressed in order to maximize the yield of natural goods from plants. Plants frequently contain trace levels of physiologically active substances. An effective extraction protocol can provide a lot of the required extracts with little alteration to their functional characteristics [12]. An efficient solvent stands out for its outstanding extraction and ability to maintain the stability of the chemical structure [9].

The amount of phenols, flavonoids, tannins, and alkaloids in plant extracts varies depending on a number of variables, including the plant component used, the solvents used, the collecting period, the extraction procedure, and the handling equipment employed [4].

Plant phenolics with antioxidant characteristics play a crucial role in avoiding oxidative stress, cytotoxicity, and cell death by scavenging free radicals or chelating trace elements and enhancing the antioxidant defenses. Phenolics, flavonoids, and tannins are the primary components of the antioxidant activity in the majority of medicinal plants [13].

Similarly, [14] reported that methanol extract of *V. officinalis* aerial parts displayed antibacterial activity against *S. aureus*.

5. Conclusion

The current study addressed the habitat features (soil conditions) where *V. officinalis* is as well as its phytochemical analysis and biological activity. Based on the present study it can be concluded that *V. officinalis* contains potential antimicrobial components that may be of great use for the development of pharmaceutical industries as an alternative therapy against various bacterial diseases.

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