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Phytochemical Screening: Antioxidant and Antibacterial Activities of *Verbena supina* L. Aqueous, Hexane and Methanol Extracts

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Abstract

This study evaluates the phytochemical constituents of *Verbena supina* L aqueous, hexane and methanol extracts as well as their antioxidant and antibacterial activities. *Verbena supina* L extracts are studied here in order to support their common use in the traditional medicine. Antioxidant activities were assessed employing 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging method, and the antibacterial activities were tested using broth microdilution method against three bacteria; *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853. The methanolic extract showed the highest antioxidant activity with IC_{50}value of 6.7±1.37 µg/mL; it was followed by the aqueous extract (IC_{50}= 9.9±1.20 µg/ml), and the hexane extract (IC_{50}=19.9±1.05 µg/mL). In addition, the *V. supina* methanolic extract showed the the strongest antibacterial activity against *S. aureus* and *E. coli* strains with a MIC value of 0.25 mg/mL and 1.75 mg/mL, respectively.

Keywords: *Verbena supina*, Antimicrobial, Antioxidant, Phytochemicals.

1. Introduction

Verbena supina L., commonly known as trailing vervain, belongs to the Verbenaceae family. It is frequently grown as small clumps, but seldom occurs as insufficient quantity to be considered a weed (Cunningham *et al.,* 2011). The species is native to the Mediterranean region. Its habitat ranges from southern and eastern Europe, northern and eastern tropical Africa, to the Middle East, Pakistan, Malaysia and Australia (Munir, 2002). The plant species of *Verbena* are used in numerous cases in traditional medicine as anti-depressants, anti-jaundice and anti-inflammatory medications, and also as diuretics and expectorants, as well as anti-influenza medications, (Al-Amier *et al.,* 2005; Wichtl, 2004). In fact, the most common species used in traditional medicine is *Verbena officinalis* (Mengiste *et al.,* 2015; Deepak and Handa, 2000; Casanova *et al.,* 2008; Tang and Eisenbrand, 1992); however, other sub species of *Verbena* are possibly interchangeable.

The phytochemical investigation and the isolated compounds of *Verbena* have shown the presence of polyphenols, flavonoids, terpenoids, glycosides, and saponins (Soares *et al.,* 2016; Abebe *et al.,* 2017; Verma and Siddiqui, 2011; Kawashty and El-Garf, 2000). Moreover, they have shown multiple biological activities such as anti-tumor (Kou *et al.,* 2013), antioxidant (Abebe *et al.,* 2017), analgesic (Abdelshafeek *et al.,* 2010) and anti-inflammatory activities (Calvo, 2006), in addition to their antidepressant (Jawaid *et al.,* 2015), antinociceptive (Braga *et al.,* 2012), neuroprotective effects (Lai *et al.,* 2006). They also are effective in enhancing the activity of nerve growth factors (Li *et al.,* 2003). Furthermore, recent research has demonstrated that the plant has anticonvulsant, anxiolytic, and sedative activities (Khan *et al.,* 2016). Such biological activity reports explain its common use in folk medicine.

The scientific literature on *Verbena supina* L., shows that its essential oil exhibits antimicrobial, antifungal and phytotoxic activities. Moreover, its flavonoids and total polyphenol content were previously studied (Al-Amier *et al.,* 2005; Kawashty and El-Garf, 2000; Dallali *et al.,* 2014), and in order to further develop the phytochemical and biological activity investigation of this plant, the aqueous, methanol and *n*-hexane extracts of *V. supina* were subjected in the current study to phytochemical screening followed by antioxidant and antibacterial activity investigations.

2. Materials and Methods

2.1. Plant Materials

All of the *V. supina* plant samples were collected from the mountains of Hebron region of Palestine during July, 2016. The plant has been botanically identified by pharmacognosist Dr. Nidal Jaradat from the Pharmacy Department at An-Najah National University. A voucher specimen Pharm-PCT-2608 has been retained in the

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herbarium of the Laboratory of Pharmacognosy. The plant was washed well several times with distilled water, and was then dried in the shade for four weeks at room temperature. After drying, the leaves were grounded into fine powder using a blender. The powder was then kept in airtight containers with proper labeling for future use.

2.2. Chemicals and Equipment

Methanol, *n*-hexane, Millon's reagent, Benedict's reagent, Sodium hydroxide, magnesium ribbon, and acetic acid were obtained from Lobachemie (India). While Trolox ((s)-(-)-6 hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid), (DPPH) 2,2-Diphenyl-1-picrylhydrazyl, chloroform, and hydrochloric acid were obtained from Sigma-Aldrich, Germany. Ninhydrin solution, Molish's reagent, sulfuric acid, and Iodine solution were obtained from Alfa-Aesar, England. $FeCl_3$ from Riedeldehan, Germany. Nutrient broth from Himedia (India). Dimethyl sulfoxide (DMSO) and antibiotics from OXOID, England. Moreover, the instruments used in the current study include: Spectrophotometer (Jenway 7135, England), Freeze dryer (Mill rock technology, model BT85, Danfoss, China), Shaker device (Memmert Shaking Incubator, Germany), Rotary evaporator (Heidolph VV2000, Germany), Grinder (Moulinex model, Uno. China), Filter papers (Whatman no.1, USA and Machrery-Nagel, MN 617), Micro-broth plate (Greiner bioone, North America), Micro-pipettes (Finnpipette, Finland), Syringe filter 0.45 µm pore size (Microlab, China), and incubator (Nuve, Turkey).

2.3. Preparation of Plant Extracts

The powdered plant material was extracted in hexane and methanol separately at a 10 % (w/v) concentration (25 g powder in 250 mL *n*-hexane or methanol) using Soxhlet apparatus for seven hours. The extracts were evaporated over a water bath at 30-40ºC, and were then kept in a refrigerator at 2-8 ºC until further use. Also, some powdered plant material was extracted in distilled water at a 10 % (w/v) concentration (25 g powder in 250 mL distilled water). The mixture was heated and stirred in a hot plate at 30º-40ºC for twenty minutes; then the extract was filtered through filter paper. The filtrate was used for the phytochemical analysis.

2.4. Antioxidant Activity

The free radical scavenging activity of the *V. supina* extracts and standard were measured according to the procedure described earlier (Jaradat *et al.,* 2015). DPPH radical solution at a concentration of 0.002 % w/v was mixed with methanol and the prepared concentrations (1, 2, 3, 5, 7, 10, 20, 30, 40, 50, 80, 100 µg/mL) from three plant extracts of 1mg/mL in methanol and standard Trolox in a ratio of 1:1:1, respectively. The solutions were left in dark incubation for thirty minutes at room temperature. Absorbance readings were recorded at 517 nm. The percentage of inhibition of DPPH activity (I %) was calculated using the following equation (Jaradat *et al.,* 2015):

$$I\ \% = ((A_{blank} - A_{sample})/ A_{blank}) \times 100$$

where: A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), A_{sample} is the absorbance value of the extract.

The antioxidant half maximal inhibitory concentration (IC_{50}) for the *V. supina* plant and Trolox standard at different concentration were plotted and tabulated, and the IC_{50} for each one of them was calculated using the BioDataFit fitting program.

2.5. Qualitative Phytochemical Analysis

Preliminary qualitative phytochemical screening of primary and secondary metabolic compounds such as proteins, starch, phenols, cardiac glycosides, saponin glycosides, flavonoids, alkaloids, steroids, volatile oils, and tannins were carried out according to the standard common phytochemical methods described by Trease and Evans (1983), and Harborne (1998) for *V. supina* entire plant.

2.5.1. Antibacterial Evaluation

Antibacterial activity of aqueous, *n*-hexane and methanolic extracts of *V. supina* were tested against four bacteria, namely (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Acinetobacter baumannii* ATCC BAA 2802) by broth microdilution method following the Clinical Laboratory Standards Institute (CLSI) recommendations (Forbes *et al.*, 2002).

An aqueous extract solution of 100 mg/mL was prepared in sterile distilled water. The *n*-hexane and methanol extracts of 178 mg/mL were prepared with 100 % Dimethyl sulfoxide (DMSO), syringe filter 0.45 µm pore size was used to sterilize the solutions. Then, the aqueous, hexane and methanol extract solutions were serially diluted 2-folds with nutrient broth in a micro-broth plate. Well number eleven was a negative control of bacterial growth, while well number twelve was for positive control of bacterial growth and contained nutrient broth. ten concentrations of aqueous, and organic extract solutions were obtained from 0.098 to 50 mg/mL and 0.174 to 89 mg/mL, respectively. Dimethyl sulfoxide (DMSO) was also serially diluted 2-fold with nutrient broth and was prepared with concentrations from 0.098 % to 50 %. The final bacterial concentration in each well (except negative control) was adjusted to 5×10^5 CFU/mL by transferring 50 µL of the 0.5 McFarland standard equivalent suspension to 10 mL of broth, then the plates were covered and incubated at 35° C for eighteen hours. Each of the bacterial isolates were examined in duplicate. Minimal inhibitory concentration (MIC) was considered when the lowest concentration of the plant extract did not allow any visible bacterial growth in the test broth.

3. Results and Discussion

3.1. Phytochemical Screening

The phytochemical characteristics of *V. supina* plant are summarized in Table 1.

It shows that proteins, tannins, flavonoids, glycosides and terpenoids were found in *V. supina* plant, whereas carbohydrates, saponins and alkaloids were absent.

Table 1. Phytochemical constituents of *V. supina*.

	Aqueous extract	Methanolic extract	*n*-hexane extract
Proteins	+	+	+
Carbohydrate	–	–	–
Phenols/Tannins	+	+	+
Flavonoids	+	+	+
Saponins	–	–	–
Glycosides	+	+	+
Steroids	-	-	-
Terpenoids	+	+	+
Alkaloids	–	–	–

3.2. Antioxidant Activity

The methanolic extract of *V. supina* has the highest free radical scavenging activity followed by the aqueous, and hexane extracts. The IC_{50} of methanol, aqueous and hexane extracts were 6.7±1.37 µg/mL, 9.9±1.20 µg/mL and 19.9±1.05 µg/mL respectively. The IC_{50} of Trolox standard reference was of 4.8±1.39 µg/mL. Detailed results are shown in Table 2

Table 2. Inhibition activity for Trolox Standard and *V. supina* entire plant.

Concentration of methanol extract DPPH\ methanol	% of inhibition by Trolox	% of inhibition by *V. supina* methanol extract	% of inhibition by *V. supina* *n*-hexane extract	% of inhibition by *V. supina* aqueous extract
1µg\mL	21.9±1.20	33.92±0.98	3.35±0.15	19.95±0.66
2 µg\mL	38.15±1.44	43.85±1.22	11.11±0.87	21.69±1.35
3 µg\mL	43.19±1.35	50.35±1.38	13.16±0.87	22.84±0.98
5 µg\mL	63.19±1.12	56.97±1.78	17.53±0.94	34.97±1.11
7 µg\mL	68.78±1.45	66.76±1.31	19.49±0.99	46.92±1.12
10 µg\mL	77.9±1.22	70.55±1.65	25.36±1.11	58.78±1.2
20 µg\mL	79.15±1.35	70.59±1.55	31.23±1.12	64.25±1.13
30 µg\mL	81.12±1.74	72.46±1.44	36.13±1.36	74.85±1.27
40 µg\mL	81.9±1.25	73.69±1.27	37.05±1.41	82.95±1.55
50 µg\mL	83.95±1.77	78.45±1.34	36.97±1.25	86.98±1.35
80 µg\mL	84.85±1.55	83.53±1.35	46.18±1.25	92.2±1.25
100 µg\mL	90.7±1.35	93.76±1.25	53.4±1.34	95.75±1.47
IC_{50}	4.8±1.39	6.7±1.37	19.9±1.05	9.9±1.20

3.3. Antibacterial Activity

The MIC values of aqueous and organic extracts of *V. supina* against examined bacterial isolates are shown in Table 3.

Table 3. Antibacterial activity of aqueous, methanol and *n*-hexane extracts of *V. supina* plant.

Bacterial strains	MIC value (mg/mL)		
	aqueous extract	methanol extracts	*n*-hexane extract
S. aureus	20	0.25	1.5
E. coli	20	1.75	3.75
P. aeruginosa	25	6.5	2.5
A. baumannii	40	6.5	1.5

The aqueous extract of *V. supina* showed antibacterial activity MIC = 20 mg/mL against *S. aureus* reference isolate, which is a Gram-positive bacterium. In addition, the *V. supina* aqueous extract was found to exhibit similar antimicrobial activities MIC = 20 mg/mL against *E. coli* Gram-negative bacterial isolates of the present study. The MIC value of the aqueous extract against *P. aeruginosa* was equal to 25 mg/mL. The Aqueous extract of *V. supina* showed a lower level of activity MIC= 40 mg/mL against *A. baumannii* Gram-negative. Obviously, the antimicrobial activity of methanol and hexane extracts of *V. supina* were stronger than the aqueous extract. Among the examined isolates, the methanolic extract showed the strongest activity against *S. aureus* and *E. coli* MIC = 0.25 mg/mL and 1.75 mg/mL respectively. It was moderately followed by the *n*-hexane extract against *S. aureus* and *A. baumannii* MIC = 1.5 mg/mL. as for the methanolic extract against *P. aeruginosa* and *A. baumannii*, MIC = 6.5 mg/mL; on the other hand, MIC of the hexane extract against *P. aeruginosa* and *A. baumannii* was 2.5 mg/mL and 1.5 mg/mL respectively. A lower level of inhibition was examined between Gram negative and Gram-positive isolates. However, the activity of both methanol and hexane extracts against Gram-negative bacteria was much higher than that of the aqueous extract. Methanolic and hexane extract activities against the bacterial isolates could be related to the outer membrane in Gram-negative bacteria but not Gram-positive bacteria.

4. Conclusion

In conclusion, the current study showed that the methanolic extracts of *V. supina* have a high content of phenolic compounds and show a high antioxidant activity, therefore, they can be used to treat several diseases that are on the rise. in which there is an increase in free radical production. Also, the *V. supina* methanolic and hexane extracts, possess significant antibacterial activity against the Gram-positive *S. aureus* and strong antibacterial activity against the Gram-negative bacterial *E. coli*. The current study also shows that the antibacterial activity of the *V. supina* methanolic and hexane extracts was stronger than that of the aqueous extract. However, further studies are still needed to identify which phytogenic phenols are responsible for the antioxidant activity of the entire plant, and to assess the way in which the phytogenic substances contribute to this activity.

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