

## *In vitro* Antimicrobial Activity of Methanolic Extract from *Varthemia iphionoides* Leaves

Moawiya A. Haddad<sup>1</sup>, Saeid M. Abu-Romman<sup>2</sup> & Ahmad S. Sharab<sup>1</sup>

<sup>1</sup> Department of Nutrition and Food Processing, Faculty of Agricultural Technology, Al-Balqa' Applied University, Al-Salt, Jordan

<sup>2</sup> Department of Biotechnology, Faculty of Agricultural Technology, Al-Balqa' Applied University, Al-Salt, Jordan

Correspondence: Moawiya A. Haddad, Department of Nutrition and Food Processing, Faculty of Agricultural Technology, Al-Balqa' Applied University, Al-Salt 19117, Jordan. Tel: 962-5-353-2519. Fax: 962-5-353-0469. E-mail: maahaddad@yahoo.com

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### Abstract

In the Mediterranean region, *Varthemia iphionoides* is commonly used in folk medicine for the treatment of gastrointestinal disorders. The present study described the antimicrobial activity of the methanolic extract of *V. iphionoides* leaves. The extract was assayed against a panel of pathogenic bacteria and fungi using agar well diffusion method. The antibacterial activity of the methanolic extract was investigated against six standard bacterial species and was found to exhibit high antibacterial activity. The most sensitive bacterium was *Klebsiella pneumoniae* ATCC 13883 followed by *Proteus vulgaris* ATCC 13315 and methicillin-resistant *Staphylococcus aureus* ATCC 95047. The least sensitive bacterium to *V. iphionoides* methanolic extract was *Escherichia coli* O157:H7 ATCC43895. Antifungal susceptibility of 13 fungal species was tested against *V. iphionoides* methanolic extract. Among the fungal species studied, *Fusarium lini* was the most sensitive and *Beauveria bassiana* was the most resistant to the extract. Good antifungal activity has been displayed by the methanolic extract of *V. iphionoides* against *Aspergillus brasiliensis*, *Aspergillus niger*, and *Aspergillus alliaceous*, *Aspergillus flavus*, *Cunninghamella echinulata*, *Gibberella fujikuroi*, *Macrophomina phaseolina*, *Cephalosporium aphidicola*, *Rhizopus stolonifer*, *Curvularia lunata* and *Cunninghamella elegans*. The observed antimicrobial potential of *V. iphionoides* indicated that this plant possesses bioactive compounds that are able to combat pathogenic microorganisms and support its traditional use in the treatment of pathogen infection.

**Keywords:** antibacterial, antifungal, methanolic extract, medicinal plant, *Varthemia iphionoides*

### 1. Introduction

The misuse of antimicrobial drugs is often associated with drug resistance in pathogenic microorganism (Cowan, 1999). Drug resistance resulted in critical health problems during the treatment of infectious disease (Mahady, 2005). The increasing public awareness of hazards associated with conventional antimicrobial drugs and the public trend of adopting natural antimicrobial agents have accelerated the use of plants and their extracts to combat microbial infections (Abreu, McBain, & Simoes, 2012; Zhang et al., 2013; Cateau, Van Bambeke, & Quetin-Leclercq, 2015).

To protect themselves against microbial and herbivory attacks, plants produce a large variety of secondary metabolites, such as, quinones, tannins, flavonoids, terpenoids, and alkaloids. These metabolites have been found to possess antimicrobial activities (González-Lamothe et al., 2009; Savoia, 2012). The concentrations of these phytochemicals are particularly very high in medicinal and aromatic plants. These plants are widely used in ethnomedicine around the world. The antimicrobial activity of medicinal plants has been reported by an enormous number of studies worldwide (Shahid et al., 2009; Wangchuk et al., 2011).

*Varthemia iphionoides* Boiss (Compositae) is a bushy perennial plant found in Mediterranean, Irano-Turanian and Saharo-Arabian regions (Al-Eisawi, 1982). Different pharmacological therapeutic properties have been reported for *V. iphionoides*. This plant is commonly used in folk-medicine in Jordan for the treatment of gastrointestinal disorders (Afifi et al., 1991). Other clinical benefits for this medicinal plant include antiplatelet

(Afifi & Aburjai, 2004), antioxidant (Al-Dabbas, Sukanuma, Kitahara, Hou, & Fujii, 2006) and antibacterial (Al-Dabbas et al., 2005; Abu-Hijleh, Jarrar, & Adwan, 2009; Masadeh, Alkofahi, Tumah, Mhaidat, & Alzoubi, 2013) activities. Moreover, it has been reported that *V. iphionoides* possesses antidiabetic activity (Gorelick, Kitron, Pen, Rosensweig, & Madar, 2011; Kasabri et al., 2013). Most recently, emerging reports have provided evidence that *V. iphionoides* displayed anticancer activity (Thoppil, Harlev, Mandal, Nevo, & Bishayee, 2013; Yarmolinsky et al., 2015).

The aim of the present study was to evaluate the antimicrobial effects of *V. iphionoides* methanolic extract on various bacterial and fungal species.

## 2. Materials and Methods

### 2.1 Plant Material and Preparation of Plant Extract

The leaves of *Varthemia iphionoides* were collected at the flowering stage in April 2014 from wild population found in Al-Salt governorate (20 km west Amman, Jordan; semi-arid region; mean annual rainfall; 350 mm). The collected leaves were thoroughly washed with distilled water and were air dried in an aerated place at room temperature. The dried leaves were stored at -18 °C in vacuum-sealed package.

Twenty-gram portions of finely-powdered leaves were extracted for 2 days with 100 ml of absolute methanol at room temperature under constant shaking of 120 rpm. The extract was then filtered twice (Whatman no. 1) and the solvent was evaporated to dryness in a rotary evaporator at 40 °C. The dried plant extract was dissolved in 0.05% dimethyl sulfoxide to a final concentration of 30 mg ml<sup>-1</sup> and sterilized by filtration through 0.45 µm millipore filters.

### 2.2 Antimicrobial Activity Test

#### 2.2.1 Test Microorganism and Microbial Culture

The methanolic extract of *V. iphionoides* was tested against 19 microorganisms. These include six ATCC bacterial species (*Salmonella typhimurium* ATCC 19430, *Escherichia coli* O157:H7 ATCC43895, Methicillin-resistant *Staphylococcus aureus* ATCC 95047, *Proteus vulgaris* ATCC 13315, *Klebsiella pneumoniae* ATCC 13883, and *Klebsiella oxytoca* ATCC 13182) and thirteen fungal species (*Curvularia lunata*, *Rhizobius stolonifer*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus brasiliensis*, *Aspergillus alliaceus*, *Fusarium lini*, *Beauveria bassiana*, *Cephalosporium aphidicola*, *Cunninghamella elegans*, *Macrophomina phaseolina*, *Cunninghamella echinulata*, and *Gibberella fujikuroi*). Bacterial strains were cultured overnight at 37 °C in a nutrient broth and fungal strains were cultured overnight at 30 °C using Soybean Casein Digest Medium.

#### 2.2.2 Standard Drugs Used for Antimicrobial Assay

Ampicillin (10 µg/disc) and vancomycin (30 µg/disc) were used as reference antibiotics against the tested bacteria. For the fungal strains, cycloheximide (250 µg/disc) and nystatin (10 µg/disc) were employed as reference antifungal agents.

#### 2.2.3 Inhibition Zone Determination by Agar-Well Diffusion Assay

The antibacterial and antifungal assays of the methanolic extract of *V. iphionoides* were carried out by the agar-well diffusion method (Perez, Paul, & Bazerque, 1990). Briefly, 100 µl of bacterial and fungal suspensions were spread uniformly over agar plates. Small wells (5 mm in diameter) were cut in the agar plates using a sterile cork borer. Fifty microliter containing 30 mg ml<sup>-1</sup> of the plant extract were added into each well. The plates were incubated for 24 h at 37 °C for the bacterial culture, and for 48 h at 30 °C for the fungal culture. Antimicrobial activity was evaluated by measuring the diameter of the inhibition zone around the well.

### 2.3 Statistical Analysis

The data were expressed as mean ± standard error (SE) of five independent replicates. One-way ANOVA test (LSD) was used to evaluate the significant differences among means at  $P < 0.05$ .

## 3. Results and Discussion

The present study investigated the *in vitro* antibacterial and antifungal activities of the methanolic extract of *V. iphionoides* using the agar well diffusion method. This method of testing the antimicrobial activity is a common standard method recommended by the Clinical and Laboratories Standards Institute (CLSI, 2009). Methanol was chosen in the present study as the extraction solvent, since it proved to be more effective than other solvents for the extraction of antimicrobial substances from plants (Chandrasekaran & Venkatesalu, 2004; Rhouma et al., 2009).

The antibacterial activity of the methanolic extract of this medicinal plant against six standard bacterial species is shown in Table 1. The methanolic extract of *V. iphionoides* displayed antibacterial effect against all tested bacterial species with inhibition zones ranging from 1.68 to 25.2 mm. The statistical analysis revealed significant differences among the different bacterial species. *Klebsiella pneumonia* (ATCC 13883), a Gram negative bacterium, showed the maximum sensitivity to the methanolic extract of *V. iphionoides* with mean diameter of inhibitory zone of 25.2 mm.

In addition, extract of *V. iphionoides* also exhibited strong antibacterial activity against *Proteus vulgaris* (ATCC 13315), methicillin-resistant *Staphylococcus aureus* (ATCC 95047), and *Klebsiella oxytoca* (ATCC 13182) with inhibition zone diameter of 21.4, 21.3, and 18.4 mm, respectively. Whereas, the methanolic extract has a fair bacteriostatic effect on *Salmonella typhimurium* (ATCC 19430) with inhibition zone diameter of 11.0 mm. Limited bactericidal activity against *Staphylococcus aureus* and *Salmonella typhimurium* were previously reported for *V. iphionoides* water extract (Afifi et al., 1991).

The antibacterial activity of the methanolic extract against *Escherichia coli* O157:H7 (ATCC43895) was not very promising. This bacterial species was resistant to both ampicillin and vancomycin antibiotics and was the least affected by the methanolic extract of *V. iphionoides*. The low sensitivity of *Escherichia coli* O157:H7 to *V. iphionoides* extract is in agreement with a previous study. It was reported that crude aqueous extract (Afifi et al., 1991) and ethanolic extract (Masadeh et al., 2013) of the aerial part of *V. iphionoides* exhibited limited antimicrobial activity against *E. coli*.

Table 1. Antibacterial activity of methanolic extract of *Varthemia iphionoides* based on agar well diffusion method

Bacterial species	Mean diameter of inhibitory zone (mm) <sup>a</sup>	Resistance to reference antibiotics <sup>b</sup>	
		Ampicillin	Vancomycin
<i>Klebsiella oxytoca</i>	18.4 <sup>b</sup> ±1.03	Sensitive	Resistant
<i>Klebsiella pneumoniae</i>	25.2 <sup>a</sup> ±0.66	Resistant	Resistant
<i>Salmonella typhimurium</i>	11.0 <sup>c</sup> ±0.71	Resistant	Resistant
<i>Escherichia coli</i> O157:H7	1.68 <sup>d</sup> ±0.21	Resistant	Resistant
<i>Proteus vulgaris</i>	21.4 <sup>b</sup> ±1.36	Resistant	Resistant
Methicillin-resistant <i>Staphylococcus aureus</i>	21.3 <sup>b</sup> ±1.63	Resistant	Sensitive

Note. <sup>a</sup> Values are given as mean ± standard error of five replicates. Means followed by different letters are significantly different at  $P < 0.05$  according to the LSD test.

<sup>b</sup> Ampicillin was tested at 10 µg and Vancomycin was tested at 30 µg. Bacterial strain is considered antibiotic resistant when the inhibition zone diameter is equal to or small than 11 mm for Ampicillin and 13 mm for Vancomycin.

The methanolic extract of *V. iphionoides* possessed antifungal activity against 13 fungal species with inhibition zones ranging from 2.0 to 19.6 mm (Table 2). *Fusarium lini* was significantly the most susceptible fungal species with inhibition zone measuring 19.6 mm in diameter. Good antifungal activity has been displayed by the methanolic extract of *V. iphionoides* against cycloheximide- and cystatin-sensitive fungi, namely, *Cunninghamella echinulata*, *Gibberella fujikuroi*, *Macrophomina phaseolina*, *Cephalosporum aphidicola*, *Aspergillus flavus*, and *Rhizopus stolonifer*. Moreover, *Curvularia lunata* and *Cunninghamella elegans* were also susceptible to methanolic extract of *V. iphionoides* with inhibition zone diameter of 13.4 and 12.0 mm, respectively. In this regards, Afifi et al. (1991) stated that flavonoids isolated from *V. iphionoides* showed antifungal activities; the compound kumatakenin exhibited potent activity against *Fusarium solani* and *Candida tropicalis*, while 3',3'-di-*O*-methylquercetin and xanthomicrol showed antifungal activities against *Fusarium solani*, *Aspergillus parasiticus*, and *Candida tropicalis*.

Three *Aspergillus* species, namely, *Aspergillus brasiliensis*, *Aspergillus niger*, and *Aspergillus alliaceus*, which showed resistance to both cycloheximide and cystatin, exhibited high sensitivity toward *V. iphionoides* methanolic extract. However, methanolic extract of *V. iphionoides* showed weak inhibition effect on the growth of *Beauveria bassiana*, because small inhibition zone (2.0 mm in diameter) was observed.

Table 2. Antifungal activity of methanolic extract of *Varthemia iphionoides* based on agar well diffusion method.

Fungal species	Mean diameter of inhibitory zone (mm) <sup>a</sup>	Resistance to reference antifungal agents <sup>b</sup>	
		Cycloheximide	Nystatin
<i>Aspergillus flavus</i>	15.2 <sup>bcd</sup> ±1.8	Sensitive	Sensitive
<i>Aspergillus brasiliensis</i>	18.8 <sup>ab</sup> ±2.1	Resistant	Resistant
<i>Aspergillus niger</i>	16.6 <sup>abcd</sup> ±1.4	Resistant	Resistant
<i>Aspergillus alliaceus</i>	16.5 <sup>abcd</sup> ±1.0	Resistant	Resistant
<i>Fusarium lini</i>	19.6 <sup>a</sup> ±1.2	Sensitive	Sensitive
<i>Rhizopus stolonifer</i>	14.6 <sup>cde</sup> ±1.6	Sensitive	Sensitive
<i>Beauveria bassiana</i>	2.0 <sup>f</sup> ±0.15	Sensitive	Sensitive
<i>Curvularia lunata</i>	13.4 <sup>de</sup> ±0.68	Sensitive	Resistant
<i>Cunninghamella elegance</i>	12.0 <sup>e</sup> ±0.89	Resistant	Resistant
<i>Cephalosporum aphidicola</i>	15.2 <sup>bcd</sup> ±1.6	Sensitive	Sensitive
<i>Macroponiues phosillai</i>	15.6 <sup>bcd</sup> ±0.51	Sensitive	Sensitive
<i>Cunninghamella echinulata</i>	17.6 <sup>abc</sup> ±0.75	Sensitive	Sensitive
<i>Gibberella fujikuroi</i>	16.7 <sup>abcd</sup> ±1.6	Sensitive	Sensitive

Note. <sup>a</sup> Values are given as mean ± standard error of five replicates. Means followed by different letters are significantly different at  $P < 0.05$  according to the LSD test.

<sup>b</sup> Cycloheximide was tested at 250 µg and Nystatin was tested at 10 µg. Fungal strain is considered antifungal-agent resistant when the inhibition zone diameter is equal to or small than 8 mm for both Cycloheximide and Nystatin.

The present results support the traditional use of *V. iphionoides* plant in treatment of pathogen infection. Different types of phenolic compounds have been previously identified in *V. iphionoides* (Abu-Romman et al., 2015). The strong antimicrobial activities of medicinal plants are associated in parts with high content of phenolic compounds (Rhouma et al., 2009). These compounds have been suggested to inhibit microbial growth by disturbing the cytoplasmic membrane, transport, electron flow, and microbial enzymes (Sikkema, De Bont, & Poolman, 1995; Gupta et al., 2012).

In addition to phenolic compounds, the antimicrobial properties of this plant can be attributed to the presence of flavonoids and essential oils (Afifi et al., 1991; Avato, Raffo, Aldouri, & Vartanian, 2004; Al-Dabbas et al., 2006). The antimicrobial properties of flavonoids have been attributed to inhibition of DNA synthesis, cytoplasmic membrane function, and energy metabolism (Mori, Nishino, Enoki, & Tawata., 1987; Tsuchiya & Inuma, 2000; Haraguchi, Tanimoto, Tamura, Mizutani, & Kinoshita, 1998). Essential oils from medicinal plants were reported to disrupt the integrity of the bacterial cell membrane and mitochondria through disintegrating membrane lipids, as a result membranes become more permeable and disrupt the cell structure (Fei, Hao, Qipeng, & Chunfang, 2011; Solórzano-Santos & Miranda-Novales, 2012).

#### 4. Conclusion

The present results showed the efficacy of the methanolic extract of *V. iphionoides* against a panel of bacteria and fungi, indicating that this plant possesses bioactive compounds that are able to combat pathogenic microorganisms. Our results, therefore, demonstrate a promising potential of including *V. iphionoides* in pharmaceutical industry for therapy of infectious diseases. However, additional studies are necessary to investigate the toxicity and side effects of the bioactive constituents of *V. iphionoides*. Further studies are required to determine the efficacy of different *V. iphionoides* extracts against various other pathogenic microorganisms. Moreover, *V. iphionoides* obtained from different geographical regions of Jordan could also be investigated; to evaluate better potential of this medicinal plant as a source of antimicrobial compounds.

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