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Original Article

Salsola kali as a Potential Source of Antibacterial Agents; A Case Study from the Southwestern Mountains of Saudi Arabia

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Abstract *- Salsola kali, a member of the family Chenopodeaceae, is a perennial bushy species that recently invaded the Southwestern mountains of Saudi Arabia, causing degradation to natural habitats. The current study aimed to screen the phytochemicals and evaluate the antibacterial activities of various parts of S. kali aqueous extracts, to examine their therapeutic potential. Qualitative phytochemical screening for Salsola kali leaves, stems, and roots aqueous extract has been conducted. Also, the antibacterial activity of each extract was determined. The phytochemical analysis of leaves, stems, and roots aqueous extracts revealed varying amounts of alkaloids, terpenoids, flavonoids, tannins and saponins. In addition, the antibacterial results demonstrated that the inhibition zones are concentration-dependent, i.e. the more concentrated extract, the more inhibition action. Also, almost in all tests, the E. coli colony was significantly high susceptible than S. paratyphi and S. aureus, respectively, towards all Salsola kali aqueous extracts, and this trend was also noticed in gentamycin tests. The current study also concluded that all aqueous extracts of Salsola kali have considerable antibacterial activity compared to the antibiotic gentamycin. Moreover, leaf extracts have higher antimicrobial activities against S. aureus, S. paratyphi and E. coli than root extracts, while the stem extracts showed lower activity against the studied organisms. The current study suggested that investigating the medicinal potentialities of unwanted invasive plants, such as S. kali, is one of the alternative approaches towards turning them from a threat to natural habitats to a promising source of beneficial compounds, including antimicrobial agents.*

Keywords *- Phytochemical screening, Antimicrobial activity, Aqueous extract, Salsola kali.*

1. Introduction

Salsola (Rutha in Arabic) is the largest genus of the family Chenopodiaceae. This genus includes over 100 salt-tolerant species occurred mainly in the arid and semi-arid regions of Africa, the Middle East, Asia and Europe [1-23]. Members of this family are typically xerophytic in the Arabian desert and are frequently reported in floristic inventories of Saudi Arabia [4]. Salsola kali is a summer annual bushy species that begins life as a typical multiple-branched bush but then turns into a spherical form. It has the tendency to invade a variety of habitats, including; rangeland, semi-arid grasslands, as well as agricultural farms, railroad, residential and industrial areas. Because of that, S. kali is considered extremely valuable as an energy crop worldwide because it adapts easily to environments with strong abiotic stresses and produces large amounts of biomass in dry lands [5-6-7]. However, this plant has medicinal importance and is used in traditional medicinal systems [8]. Nine natural products were identified in leaf and stem extracts of S. kali, known for their biological activities, which seem to be strongly related to different organs and developmental stages. Among those nine compounds, two alkaloids: salsoline and fraxidin, were reported through phytochemical tests using the HPLC technique [9]. S. villosa, another species belonging to the family Salsola, which is growing widely in the North of Saudi Arabia, were studied and investigated. The biological activities of two new bioactive compounds extracted from this plant were studied. Those two new natural compounds were; secondary cyclic alcohol and biphenylpropanoid, which were isolated from bioactive chloroformic extract of the aerial parts of S. villosa. Those compounds are assumed to be responsible for the

plant's antibacterial properties [10]. The volatile fractions of S. vermiculata leave, roots and stems were determined by gas chromatography (GC). The major compounds of leaves volatile fraction were carvone (52.2%) and β-caryophyllene (5.8%). The major constituents of the root's volatile fraction were carvone (49.9%) and cumin aldehyde (4.4%). The stem's volatile fraction was dominated by carvone (53%), limonene (17.4%) and linalool (11.3%)[11-12-20].

Plants are known to contain innumerable biologically active compounds, which possess therapeutic properties including; antibacterial, antitumor, antiviral, anti-inflammatory, wound healing and cytotoxicity. Therefore, phytochemical components of plants play an important role in conventional medicine[6]. The antimicrobial activity of Salsola vermiculata leaves, roots and stem extracts was evaluated toward Staph. aureus, Enterococcus faecalis, E. coli, Pseudomonas aeruginosa and Acinetobacter baumanii. The ethanol extract of roots was found to be the most active against S. aureus at 0.28 mg/ml. However, E. coli and P. aeruginosa were the most resistant bacteria [11-12]. 15 species from Chenopodiaceae have been analyzed for their chemical constituents: alkaloids, anthraquinones, coumarins, flavonoids, saponins, sterols and terepenes and tannins were detected. The antimicrobial activity of these plants was tested in two concentrations against Gram-negative E. coli and Ps. aeruginosa and Gram-positive Staphy. aureus and Bacillus subtilis. The results of ethanol extract and cream showed the greatest antimicrobial activity compared to other extracts, creams, and formulations[13-14-15].

Salsola kali has recently invaded the Southwestern mountains of Saudi Arabia, causing degradation to natural habitats. Thus, this species must be either removed or exploited as a source of effective phytoconstituents with therapeutic properties, including antibacterial. Therefore, the current study aimed to screen phytochemicals and evaluate the antimicrobial activities of different parts of Salsola kali aqueous extracts collected from the Al-Baha region, Saudi Arabia.

2. Materials and Methods

2.1. Plant Material

Fresh Salsola kali specimens were collected from the Al-Baha region in southwest Saudi Arabia. Leaves stems, and roots were chopped and spread on a tarp and allowed to air dry. Then, each of the dry, coarsely chopped plant materials was further ground to pass through a 2.4 mesh screen using a Gehl Mix-All model 55 (Gehl Company, West Bend, WI, USA). After processing, each of the ground plant materials was stored in plastic bags away from direct sunlight at ambient temperature for future use.

2.2. Preparation of Aqueous Extracts

For aqueous extraction, 20 g of each air-dried powder was added to 100 ml of distilled water and incubated for 24 hours on the shaker. Then it was filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 10 minutes, and the supernatant was collected. This procedure was repeated twice; after 6 hours, the supernatant was collected at an interval of 2 hours, pooled together and concentrated on making the final volume one-fourth of the original volume (stock solution).

2.3. Phytochemical Screening

The qualitative phytochemical screening for Salsola kali leaves, stems, and roots aqueous extract has been conducted based on standard methods of analysis described by Kehail et al. (2017). [16].

Test for the presence of alkaloids: In a test tube, 3 ml of each extract and 2 drops of Dragendoff's reagent were added. The formation of orange-red precipitation proved the presence of alkaloids.

Test for the presence of flavonoids: In a test tube, 4 ml of each extract, a piece of magnesium ribbon and a drop of concentrated HCl were added. The red to crimson color detected the presence of flavonoids.

Test for the presence of saponins: In a test tube, 5 ml of each extract was shaken vigorously for 2 minutes. The presence of saponins was indicated by the formation of froth that lasted for some minutes.

Test for the presence of tannins: In a test tube, 2 ml of each extract and 3 drops of 5% ferric chloride ($FeCl_3$) solution were added. The appearance of green, black or blue colour detected the presence of tannins.

Test for the presence of terpenoids: In a test tube, 5 ml of each chloroform extract and 5 ml of concentrated sulphuric acid were carefully added. The formation of a red-brown layer indicated the presence of terpenoids.

2.4. Antibacterial Activities of Plant Extracts

The antibacterial activity of each extract was determined by the agar diffusion method. Freshly isolated colonies of S. aureus, S. paratyphi and E. coli were suspended in sterile saline to get a turbidity of 0.5 McFarland standards. 0.1 ml of this suspension was spread aseptically on a sterile Muller Hinton agar medium (Hi media). The wells of 6 mm diameter were bored by sterile cork borer. 0.2 ml of each extract (100 mg/ml in 10% Dimethyl sulfoxide; DMSO was added to the wells. It was allowed to diffuse by keeping it in freeze for 20 minutes. 10% DMSO in one of the wells was used as the negative control. After diffusion of each extract, the plates were incubated at 37oC for 24 hours. The inhibition zones were measured in mm. For each extract, three replicates were maintained.

Determination of minimum inhibitory concentration (MIC)

The tube dilution method was done to determine the minimum inhibitory concentration of each extract. A series of two-fold dilutions of each extract ranging from 10 mg/ml S. aureus, S. paratyphi and E. coli to 0.3 mg/ml was made in Muller Hinton broth. 0.1 ml suspension of S. aureus, S. paratyphi and E. coli matched to 0.5 McFarland standard was seeded into each dilution. Two controls were maintained for each test batch. These controls included tubes containing extract and growth medium, without inoculum and organism control, i.e. tubes containing the growth medium and inoculum. The tubes were incubated at 37oC for 24 hours and checked for turbidity. Minimum inhibitory concentration was determined as the highest dilution of the extract that showed no visible growth.

3. Data Analysis

The qualitative phytochemical screening results were presented as detected in low concentration (+) and detected in more concentration (++) in the special table. At the same time, the inhibition zone diameters (mm) were represented in (Mean +SE), and the least significant difference (LSD) was applied to these values to determine the significant level as letters.

4. Results and Discussion

4.1. Results

4.1.1. Phytochemical Analysis

Table (1) shows the qualitative phytochemical analysis of Salsola kali leaves, stem and roots. Varying amounts of alkaloids, terpenoids, flavonoids, tannins and saponins were detected in all tested plant parts. Medium precipitation of alkaloids (++) was present in S. kali leaves compared to low precipitation (+) for the other phytochemical compounds. Also, similar amounts of these compounds were detected in root extracts. At the same time, extracts of stems exhibited medium amounts (++) of both alkaloids and terpinoids compared to low amounts (+) for the rest of the phytochemicals.

4.1.2. Antibacterial Activity

Table (2) shows that the leaves aqueous extract of Salsola inhibited the growth of Staph—aureus by 6.3±0.12 mm and 15.01±0.45 mm, respectively, at concentrations of 25 µl and 50 µl. However, the same concentrations inhibited the growth of S. paratyphi by 14.3±0.67 mm and 18.19±0.17 mm, following the same order. The growth of E. coli was inhibited by 16.0±0.48 mm and 21.16±0.38 mm, respectively, at concentrations of 25µl and 50µl. The inhibition zones of the antibiotic gentamycin (reference control) were 32, 38 and 44 mm in Staph—aureus, S. paratyphi and E. coli, respectively, at a concentration of 50 µg/ml.

Table (3) shows that the stem aqueous extracts of Salsola inhibited the growth of Staph. aureus at concentrations of 25µl and 50µl by 5.3±0.22 mm and 8.03±0.31 mm, respectively. However, it inhibited the growth of S. paratyphi at the same order of concentrations by 9.3±0.44 mm and 12.02±0.09 mm. It also inhibited the growth of E. coli by 9.01±0.25 mm and 14.11±0.43 mm, following the same order of concentrations. The inhibition zones of gentamycin were 32, 38 and 44 mm in Staph—aureus, S. paratyphi and E. coli, respectively.

Table (4) shows the roots of aqueous extracts of Salsola spp. Inhibited the growth of Staph. aureus at concentrations of 25µl and 50µl by 11±0.15 mm and 22.03±0.77 mm, respectively. However, it also inhibited the growth of S. paratyphi at the same concentrations by 12.04±0.55 mm and 19.16±0.31 mm, respectively, and the growth of E. coli by 17±0.48 mm and 27.05±0.76 mm, following the same order of concentrations. graphics

Table 1. The qualitative phytochemical screening of Salsola spp

Plant parts	Alkaloids	Terpenoids	Flavonoids	Tannins	Saponins
Leaves	++	+	+	+	+
Stem	++	++	+	+	+
Roots	++	+	+	+	+

Table 2. Antibacterial activity (mm) of S. kali leaves aqueous extract compared to gentamycin. Different superscript letters indicate means that are significantly different at level (p<0.05).

Organism	Inhibition Zone at 25 µl	Inhibition Zone at 50 µl	Gentamycin (control) at 50 µg/ml
S. aureus	6.3 ± 0.12^{c}	15.01 ± 0.45^{c}	32^{c}
S. paratyphi	14.3 ± 0.67^{b}	18.19 ± 0.17^{b}	38^{b}
E. coli	$16.\pm0.48^{a}$	21.16 ± 0.38^{a}	44^{a}

Table 3. Antibacterial activity (mm) of Salsola stem aqueous extract compared to gentamycin. Different superscript letters indicate means that are significantly different at level (p<0.05).

Organism	Inhibition Zone at 25 µl	Inhibition Zone at 50 µl	Gentamycin (control) at 50 µg/ml
S. aureus	5.3±0.22[b]	8.03±0.31[c]	32[c]
S. paratyphi	9.3±0.44[a]	12.02±0.09[b]	38[b]
E. coli	9.01±0.25[a]	14.11±0.43[a]	44[a]

Table 4. Antibacterial activity (mm) of Salsola roots aqueous extract compared to gentamycin. Different superscript letters indicate means that are significantly different at level (p<0.05).

Organism	Inhibition Zone at 25 µl	Inhibition Zone at 50 µl	Gentamycin (control) at 50 µg/ml
S. aureus	11.0±0.15c	22.03±0.77c	32[c]
S. paratyphi	12.04±0.55b	19.16±0.31b	38[b]
E. coli	17±0.48a	27.05±0.76a	44[a]

4.2. Discussion

Results of the screening of the content of *Salaola kali* different parts of phytochemicals showed varying amounts of alkaloids, terpenoids, flavonoids, tannins and saponins. Similar findings were found with Salsola imbricata Forssk, which revealed the presence of anthraquinones, reducing sugar, tannins, saponins, flavonoids, alkaloids and cardiac glycosides [14-23]. Also, phenolic contents in S. kali leaves, stems, and roots were investigated in another study. A maximum of total phenol contents in the photosynthetic organs was reported, followed by stems and roots, respectively. Also, flavonoids and condensed tannins contents were similar to phenolic contents[8].

The antibacterial results of the current study demonstrated that the inhibition zones are concentration-dependent, i.e. the more concentrated extract, the more inhibition action. Also, almost in all tests, the E. coli colony was significantly more susceptible than S. paratyphi and S. aureus, respectively, towards all Salsola kali aqueous extracts. This trend was also noticed in gentamycin tests. Thus, the current study also concluded that all aqueous extracts of *Salsola kal*i have considerable antibacterial activity compared to the antibiotic gentamycin. Moreover, leaf extracts have higher antimicrobial activities against *S. aureus, S. paratyphi and E. coli* than root extracts, while the stem extracts showed lower activity against the studied organisms. Plants are known to have rich content of biologically active compounds, which possess therapeutic properties including; antibacterial, antitumor, antiviral, anti-inflammatory, wound healing and cytotoxicity. For example, in Saudi Arabia, two new bioactive biphenyl-propanoids from the roots of Salsola imbricata were investigated [15]. Also, the antibacterial activity of the Et-OAc extract of *Salsola villosa* roots was assessed against three Gram-positive bacteria; *S. aureus, S. epidermidis* and *Micrococcus luteus* and three Gram-negative bacteria*; E. coli, Pseudomonas aeruginosa* and *Salmonella typhimurium* [9].

In addition, crude methanol extract of Salsola kali has been assessed and was found to be highly effective against *S. mutans, S. aureus, B. subtilis and S. pneumoniae* while it showed moderate antibacterial activity against.

Meanwhile, it inhibited the growth of *S. lutae and E. coli* [5-21-22]. Such a result is similar to the result obtained by the current study. Another study was conducted in Tunisia, where nine salt marsh plants belonging to the Chenopodiaceae family were collected and investigated for their phytochemical contents. Results proved that many species were adapted to saline soils since they may contain phytochemical compounds with fungicidal properties[16].

5. Conclusion

The qualitative phytochemical analysis of Salsola kali leaves, stem and roots: alkaloids, terpenoids, flavonoids, tannins and saponins. In addition, the current study's antibacterial results demonstrated that the inhibition zones are concentration-dependent, i.e. the more concentrated extract, the more inhibition action. Also, almost in all tests, the E. coli colony was significantly high susceptible than S. paratyphi and S. aureus, respectively, towards all Salsola kali aqueous extracts, and this trend was also noticed in gentamycin tests. Thus, the current study also concluded that all aqueous extracts of Salsola kali have considerable antibacterial activity compared to the antibiotic gentamycin. Moreover, leaf extracts have higher antimicrobial activities against S. aureus, S. paratyphi and E. coli than root extracts, while the stem extracts showed lower activity against the studied organisms. Therefore, investigating the medicinal potentialities of unwanted invasive plants, such as S. kali, is one of the alternative mechanisms towards turning them from a threat to natural habitats to a promising source of beneficial compounds, including antimicrobial agents.

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