Research article

Phytochemical analysis and antibacterial potential of *Onosma hispidium* and *Alcea rosea*

Saima Nazir1,5, Mir Kaisar Ahmad2, Fasil Ali3, Zubair-Ul-Nazir4, Showkat Ahmad Ganie5

1Department of Biotechnology, Mewar University, Chittorgarh, 312901, Rajasthan, India
2Department of Biochemistry, Government Medical College Baranwala, 193101, Jammu & Kashmir, India
3Department of Studies and Research in Biochemistry, Mangalore University, 571232, Karnataka, India
4Bangladesh Medical College, Dhaka, 1209, Bangladesh
5Department of Clinical Biochemistry, University of Kashmir, Srinagar, 190006, Jammu & Kashmir, India

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Corresponding author: Saima Nazir. Email: mirsaima13@gmail.com

**ABSTRACT**

**Introduction and Aim:** Traditional medicine heavily relies on use of medicinal plants to treat a variety of infectious disorders in humans. The medicinal herbs like *Onosma hispidium* and *Alcea rosea* have been traditionally used for the variety of clinical disorders like jaundice, diabetes, malaria, rheumatism and have been used as laxative, anthelmintic, disorder of blood, disease of eyes, bronchitis, abdominal pain, antibacterial and as a wound healer. The objective of this research was to see how active aqueous, ethyl acetate and methanolic extracts were at preventing infection by *Onosma hispidium* and *Alcea rosea*, a traditionally used medicinal plants with multiple therapeutic properties.

**Materials and Methods:** The susceptibility of microbial strains of the plant extracts was determined using agar diffusion method. The bacterial strains tested were *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Escherichia coli*. Standard screening procedures were used in phytochemical screening.

**Results:** Both the methanol, ethyl acetate, and aqueous extracts showed a dose-reliant rise in antibacterial activity. Among the plants screened, in *Onosma hispidium* the highest antibacterial activity was exhibited by aqueous extract with *Pseudomonas aeruginosa* (25±1.88) followed by *Staphylococcus aureus* (22 ± 0.22 mm) and *Klebsiella pneumoniae* (20.21±1.01) at the concentration of 100 mg/ml respectively. The antibacterial activity of the methanolic extract was highest against Escherichia coli and Proteus vulgaris with a zone of inhibition of (25±1.42mm) and (11±0.19mm) respectively, while in case of *Alcea rosea*, the ethyl acetate extract exhibited the highest antibacterial activity with *Escherichia coli* (28±1.56) followed by *Staphylococcus aureus* (25 ± 0.58 mm). *Klebsiella pneumoniae* (18±0.74) and *Proteus vulgaris* (13±0.12) at the concentration of 100 mg/ml respectively. Phytochemical showed the plants are rich in various secondary metabolites like alkaloids, saponins, flavonoids, phenols, terpenoids and volatile oils.

**Conclusion:** The plants contain novel compounds with broad spectrum antibacterial properties. The separation and classification of these new compounds could lead to the formation of beneficial antimicrobials to fight pathogenic infections.

**Keywords:** Microbial strains; phytochemicals; anti-microbial; *Onosma hispidium*; *Alcea rosea*.

**INTRODUCTION**

Plants have a lot of promise when it comes to developing new medicines for human use. Modern medicine plants contain a wide range of constituents that can be employed to treat chronic and infectious diseases. According to a World Health Organization survey, conventional medicine is used by more than 80% of the world’s population for primary health care (1). Scientists began developing synthetic antimicrobials derived from microbial sources after the discovery of antibiotics at the turn of the twentieth century. Ironically, the widespread use of synthetic drugs in humans and other species, as well as their increasing prevalence in soil, water, and food, has exacerbated the serious problem of antimicrobial resistance (2). Antimicrobial resistance has turned many antibiotics useless today, which is a significant public health problem. Synthetic antibiotics or miracle drugs are no longer as powerful as they once were considered for more than 50 years of use. Multiple drug resistant strains are on the rise at an alarming rate. In reality, antibiotics are becoming immune to the majority of bacterial infections around the world (3). New synthetic antibiotics are also being generated at a much lower rate. Furthermore, synthetic antibiotics have been linked to host side effects such as hypersensitivity, immune suppression and allergic reactions (4). As a result, medicinal plants must be screened for biological activity. Furthermore, resistant strains are constantly evolving, necessitating the quest for and discovery of new drugs to treat diseases (5).

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Pathogenic bacteria have always posed a significant threat to human and animal health. Numerous infectious diseases like tuberculosis, AIDS, syphilis, candidiasis, aspergillosis, amoebiasis, poliomyelitis, etc. are caused by microbes such as bacteria, viruses, fungi, or protozoans. Infectious diseases are in reality, the world’s second leading cause of death (6). Infectious bacteria have contaminated humans since the dawn of time and continue to do so today. Microbes like *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Escherichia coli* cause dermal infections, sepsis, upper and lower respiratory infections. Gynaecological diseases are also caused by *E. coli*. *Staphylococcus aureus* causes intra-abdominal infections, orthopaedic, dermal and pulmonary ailments/ diseases. *Proteus vulgaris* is an opportunistic pathogen responsible for triggering urinary tract infections (UTI) and wound infections (7). *Bacillus subtilis* is an opportunistic pathogen that produces toxins that can cause food poisoning (8).

India is a medicinal plant varietal emporium and one of the world's richest countries in terms of medicinal plant genetic capital. It has a diverse topography and climate, which has an impact on the vegetation and floristic composition of the region. In addition, the agro-climatic conditions are favourable for the introduction and domestication of new exotic plant varieties (9). Antimicrobial phytochemicals, such as flavonoids, saponins, tannins, alkaloids, phenols, terpenes, and terpenoids, are abundant in medicinal plants (10). Herbal extracts have been shown to have antimicrobial properties against a variety of pathogenic microbes in scientific studies (11, 12, 13). Man has used various sections of plants in the treatment and prevention of various ailments since the dawn of time (13). It is worth noting that antimicrobial phytoconstituents are becoming more common as a means of controlling pathogenic microorganisms.

The present study was conducted to evaluate the antimicrobial potential of the plant *Onosma hispidium* and *Alcea rosea* against some bacterial strains. *Onosma hispidium*, a member of the *Onosma* genus in the Boregineaceae family, is a 70 cm tall, perinnael herb with a prominent tap root that is historically known as Ratanjot. It is distributed in India, Pakistan, Afghanistan and China which is used traditionally for laxative, anthelmintic, disorder of blood, disease of eyes, bronchitis, abdominal pain, antibacterial and as wound healer (14). This plant's antitussive, anticholinesterase inhibitor, lipoxygenase inhibitor, antimicrobial, and antidiabetic properties have all been scientifically proven (15). In India, it is used as a spice to add colour and flavour to a variety of curries (16).

Hollyhock, or *Alcea rosea*, is a popular ornamental plant of Malvaceae family, with large showy blossoms in a variety of colours. It is thought to have originated in China or Southeast Asia's tropical regions. It is used to treat kidney and uterine inflammation, digestive tract infections with vomiting and diarrhoea, kidney and urinary tract infections, jaundice, malaria, rheumatism, and snake bites in herbal medicine (17). The stated scientific properties of *A. rosea* are antimicrobial, hypoglycemic activity in diabetic mice, immunomodulatory property, analgesic and anti-inflammatory activity (18).

**MATERIALS AND METHODS**

**Collection and identification of plant material**

The whole plant of *Onosma hispidium* and *Alcea rosea* were collected from Badamwari Srinagar area of Jammu and Kashmir during the month of June, authenticated by the Centre of Plant Taxonomy, Department of Botany, University of Kashmir, and authenticated by Akhter Hussain Malik. A reference specimen has been preserved in the herbarium under reference number KASH-Bot/ku/AR-705-IA and KASH-Bot/ku/OH-706-IA.

**Preparation of extracts**

The authentically identified plant material was shade dried under room temperature at 30 ± 2°C. The dried plant material was ground into powder using mortar and pestle and sieved with a sieve of 0.3mm aperture size. The dried material obtained was extracted using different solvents like hexane, ethyl acetate, absolute ethanol, methanol, and aqueous solvents by using Soxhlet extractor at a temperature of 60-80°C. The extracts were then concentrated with the help of rotary evaporator under reduced pressure, and the solid extract was stored in refrigerator for further biochemical analysis.

**Test for micro-organisms**

Five bacterial strains including Gram positive bacteria namely *Staphylococcus aureus* (MTCC-2940) and four Gram negative bacteria namely *Proteus vulgaris* (MTCC-426), *Klebsiella pneumonia* (MTCC-139), *Escherichia coli* (MTCC-739) and *Pseudomonas aeruginosa* (MTCC-424) were employed for antibacterial assay. The Bacterial strains were obtained from Microbial Type Culture Collection, Institute of Microbial Technology (IMTECH), Chandigarh, India. Bacterial strains were maintained by sub-culturing them on Mueller Hinton Agar (Himedia) after every fifteen days and then stored at 4°C. Gentamycin discs were obtained from EOS Laboratories, India and served as positive control for antibacterial assay. 10% Dimethylsulfoxide (Himedia) was used as negative control.

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Antibacterial assay

Antibacterial assay for aqueous, ethyl acetate and methanolic extracts was performed by agar well diffusion method as described by (19) with some modification. 100µl of standardized inoculum (0.5 Mc Farland) of each test bacterium was inoculated on molten Mueller Hinton Agar (Himedia), thoroughly mixed and then poured into sterile plates and allowed to solidify inside the laminar hood, to yield a uniform depth of 4 mm. 100mg/ml of plant extracts, prepared in 10% dimethyl sulfoxide (DMSO) were loaded into different peripheral wells. Genta mycin (10 µg/disc) disc was placed at the centre of each petri plate and served as positive control, while as 10% dimethyl sulfoxide was used as negative control. The petri plates were then incubated at 37º C for 18 to 24 hours in an incubator. The plates were then observed for the zones of inhibition. Antibacterial potential was evaluated by measuring the diameters of zones of inhibition in millimetres (mm) using a standard measuring scale.

Phytochemical analysis

Preliminary phytochemical screening of aqueous and methanolic extracts of Onosma hispidium and Alcea rosea was performed according to methods described by Harborne (20).

Table 1: Showing preliminary phytochemical screening of aqueous and methanolic extracts of Onosma hispidium

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Aqueous extract</th>
<th>Methanol extract</th>
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<tbody>
<tr>
<td>Tannins</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Flavonoids</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Phenols</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Phlobtannins</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Cardenolides</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Volatile oils</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Anthraquinone</td>
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Table 2: Preliminary phytochemical screening of methanol and ethyl acetate extracts of Alcea rosea

<table>
<thead>
<tr>
<th>Phytochemicals</th>
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<th>Ethyl acetate</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Flavonoids</td>
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<td>+</td>
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<tr>
<td>Saponins</td>
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<td>+</td>
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<tr>
<td>Steroids</td>
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</tr>
<tr>
<td>Terpenoids</td>
<td>+++</td>
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<td>Alkaloids</td>
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<tr>
<td>Phlobtannins</td>
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<tr>
<td>Cardiac glycosides</td>
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<tr>
<td>Cardenolides</td>
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<tr>
<td>Volatile oils</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Anthraquinone</td>
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Statistical analysis

The values of zones of inhibition was expressed as mean ± standard deviation (SD) of three independent experiments. The results were analysed using SPSS (version 12.0) and then evaluated by one way ANOVA followed by Bonferroni t-test. The statistical significance was considered at a value of P<0.05.

RESULTS

Phytochemical analysis

The phytochemical analysis of different extracts of Onosma hispidium revealed the presence of maximum phyto-constituents in polar solvent (Table 1). Saponins and phlobtannins were maximally present in methanol extract however absent in aqueous extract. Phenols, steroids, terpenoids, cardiac glycosides and cardenolides were maximally present in methanolic extracts but moderately present in the aqueous extract. Besides moderate content of alkaloids was found in methanol and aqueous extracts. Volatile oils and anthraquinone were absent in the methanol and aqueous extracts.

Alcea rosea also showed maximum phyto-constituents (Table 2). The results show the presence of maximum content of flavonoids, tannins, terpenoids, alkaloids, volatile oils. Saponins were found low in both extracts. Steroids were present at low concentration in methanol extract. Cardenolides were found to be absent in both the extracts. Volatile oils were observed high in methanol extract, phenols and phlobtannins were present in equal amount.
Antibacterial Activity

In the plant extracts of *Onosma hispidium* and *Alcea rosea*, the antibacterial activity of both the plants differed with the type of bacterial strain and the extract used. The aqueous extract of *Onosma hispidium* was found more effective against *Staphylococcus aureus* (22 ± 0.22; Fig. 1) *Klebsiella pneumoniae* (20.21±1.01; Fig. 2) and *Pseudomonas aeruginosa* (25±1.88; Fig. 5) at the concentration of 100 mg/ml respectively, while the methanolic extract was found most effective against *Proteus vulgaris* (11±0.19; Fig. 3) and *Escherichia coli* (25±1.42; Fig. 4). However, in case of *Alcea rosea*, the ethyl acetate extract was found more potent against *S. aureus* (25 ± 01.58; Fig. 1) *Klebsiella pneumoniae* (18±0.74; Fig.2) *Proteus vulgaris* (13±0.12; Fig. 3) and *Escherichia coli* (28±1.56; Fig. 4) and Methanolic extract of *Alcea rosea* was more effective against *Escherichia coli* (19±0.72; Fig. 4) and *Pseudomonas aeruginosa* (21±0.24; Fig. 5). The results were compared to a standard antibacterial drug, Gentamycin (G) which showed the zone of inhibition of 30 ± 1.45mm against *Klebsiella pneumoniae*, 25 ± 1.10 mm against *Proteus vulgaris*, 25 ± 1.58 mm against *Pseudomonas aeruginosa*, 40 ± 1.56 mm against *Escherichia coli* and 32 ± 1.97 mm against *Staphylococcus aureus* respectively. 10% DMSO (negative control) showed no activity against any of the tested bacterial strains.

Fig. 1: Showing the antimicrobial activity of different extracts of *Alcea rosea* and *Onosma hispidium* against *S. aureus* [1-AR (Ethyl acetate), 2-OH (Aqueous), 3-AR (Ethyl acetate Root), 4-OH (methanol), 5-AR 50°C. Gentamycin (G) was used as positive control (Centre)]

Fig. 2: Showing the antimicrobial activity of different extracts of *Alcea rosea* and *Onosma hispidium* against *K. pneumoniae* [1-AR (Ethyl acetate), 2-OH (Aqueous), 3-AR (Ethyl acetate Root), 4-OH (methanol), 5-AR 50°C. Gentamycin (G) was used as positive control (Centre)]

Figure 3: Showing the antimicrobial activity of different extracts of *Alcea rosea* and *Onosma hispidium* against *P. vulgaris* [1-AR (Ethyl acetate), 2-AR (Ethyl acetate Root), 4-OH (methanol), 5-AR 50°C. Gentamycin (G) was used as positive control (Centre)]

Fig. 4: Showing the antimicrobial activity of different extracts of *Alcea rosea* and *Onosma hispidium* against *E.coli* [1-AR (Ethyl acetate), 2-AR (Aqueous), 3-AR (Ethyl acetate Root), 4-OH (methanol), 5-AR 50°C. Gentamycin (G) was used as positive control (Centre)]

Fig. 5: Showing the antimicrobial activity of different extracts of *Alcea rosea* and *Onosma hispidium* against *P.aeruginosa* [1-AR (Ethyl acetate), 2-OH (Aqueous), 3-AR (Ethyl acetate Root), 4-OH (methanol), 5-AR 50°C. Gentamycin (G) was used as positive control (Centre)]
DISCUSSION

In the present study, the antimicrobial activity of aqueous and methanol extract of *Onosma hispidum* and the methanol and ethyl acetate extract of *Alcea rosea* was proven to be effective against most of the bacterial strains. However, the methanol and ethyl acetate extract of *Alcea rosea* showed no zone of inhibition against *K. pneumoniae*. As stated by several reports, this may be because different phytochemicals appear in different solvents due to their relative polarities/solubility differences (21). The antimicrobial activity of both plant extracts against various clinical bacteria strains backed the scientific legitimacy of the plant's conventional use as a medicine. The presence of soluble phenolic and polyphenolic compounds in the extracts can explain the inhibition of a maximum of five bacterial strains by methanol, aqueous, and ethyl acetate extracts. The results correspond to that of a recent report in which it has been shown that the was that the methanol extract of *Nepeta cataria* inhibited the growth of all bacterial test organisms, implying that the extracts antimicrobial activity could be due to certain phenolic components (22). The absence of antibacterial activity in some of the extract concentrations is not surprising, as many plant extracts have been shown to be ineffective against some test species at lower concentrations, which may be due to the existence of lower levels of antimicrobial compounds. The antibacterial effects of the extracts could be clarified by disruption of the bacterial membrane structure's permeability barrier (23). In terms of microbe resistance, *Escherichia coli* and *Pseudomonas aeruginosa* were found to be the most susceptible bacterial strains in both plants. The different solvent extracts of *Melia azedarach L* also shows anti-microbial activity too, has been reported earlier (24). The phytochemical analysis of the plant extracts showed the presence of alkaloids, flavonoids, saponins, tannins, phenols, cardiac glycosides, and volatile oils. Our findings are very similar to those reported earlier when analysing the phytochemical content of various plant extracts of *Punica granatum L* (25). The existence of any of these phytoconstituents in the plant can confer antimicrobial activity through a variety of mechanisms, including disruption of cell membranes, inhibition of cell wall formation, inactivation of microbial adhesins, suppression of enzymes, or blocking of nucleic acid synthesis (26). Furthermore, studies have shown that phytochemical compounds can influence various parameters such as efflux pumps, beta-lactamase enzymes, resistance plasmids, and bacterial gene transposition to disrupt the activities of multiple drug resistant microbes (27).

CONCLUSION

The present study confirms that the plant *Onosma hispidum* and *Alcea rosea* possess significant antimicrobial components that may be of abundant use for pharmaceutical industries as a therapy against various diseases. The methanol, ethyl acetate and aqueous extracts of *Onosma hispidum* and *Alcea rosea* possess significant inhibitory effect against tested pathogens. These extracts may be screened for pure compounds that could be used directly or as a base for the production of new antibiotics. The findings back up the folklore argument, as well as the discovery of new antimicrobial drugs derived from both plants.
ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that no competing interests exist.

REFERENCES


