Antimicrobial activity of leaf extracts of Jurinea dolomiaea plant against clinical and phytopathogenic bacteria.

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Abstract
The ethnobotanical efficacy of Jurinea dolomiaea plant is examined using agar disc diffusion method against clinical bacteria (Escherichia coli and Staphylococcus aureus) and phytopathogenic bacteria (Xanthomonas vesicatoria and Ralstonia solanacearum). Leaves were extracted using different solvents such as methanol, ethanol, ethyl acetate and chloroform. Among treatments, maximum in vitro inhibition was scored in methanol extract of which offered inhibition zone of 10, 9, 12 and 12 mm against E. coli, S. aureus, X. vesicatoria and R. solanacearum, respectively, followed by chloroform extract of the same plant leaf with inhibition zone of 8, 4, 4 and 4 mm, respectively. The minimum inhibitory concentration (MIC) value for the clinical bacteria ranged between 0.35 to 4.0 mg/ml and 0.25 to 4.0 mg/ml for phytopathogenic bacteria when tested with all four solvents extracts of J. dolomiaea.

Introduction
Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products and used in the traditional systems of medicine. Thus it is a logical approach in drug discovery to screen traditional natural products. Approximately 20% of the plants found in the world have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced on the market are obtained from natural or semi synthetic resources. It has been reported that between the years 1983 and 1994 (Cragg et al., 1999), the systematic screening of antibacterial plant extracts represents a continuous effort to find new compounds with the potential to act against multi-resistant bacteria. Several workers throughout the world have carried out antimicrobial studies on some medicinal plants including Betula pendula (Mukhtar et al., 2002) and Ageratum houstonianum (Bowers et al., 1976). According to World Health Organization (Santos et al., 1995) medicinal plants would be the best source to obtain a variety of drugs.

Current advancements in drug discovery technology and search for novel chemical diversity have intensified the efforts for exploring leads from Ayurveda the traditional system of medicine in India. Ayurvedic system of medicine has its long history of therapeutic potential. The use of plant extracts and phytochemicals both with known antimicrobial properties is of great significance, in the past few years a number of antimicrobial activities have been conducted worldwide to prove antimicrobial activities from medicinal plants (Alonso-Paz et al., 1995; Nascimento et al., 1990). For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. According to World Health Organization (Santos et al., 1995) medicinal plants would be the best source to obtain a variety of drugs. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are a part of the essential oils (Jansen et al., 1987) as well as tannin (Saxena et al., 1994).

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas et al., 2003). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (Benkeblia, 2004). Green plants represent a reservoir of effective chemotherapeutants and can provide valuable sources of natural pesticides (Mahajan and Das, 2003). Biopesticides has been suggested as an effective substitute for chemicals (Kapoor, 2001). Reports are available on the use of several plant by-products, which posses antimicrobial properties, on several pathogenic bacteria and fungi (Bylka et al., 2004; Shimpi and Bendre, 2005; Kilani, 2006). Here, we evaluate the potential of several plant extracts for antibacterial activity against important human pathogenic and phytopathogenic bacteria.

MATERIALS AND METHODS
Plant material
Fresh leaves of Jurinea dolomiaea was collected from Hemalian region of upper Danchigam in south Kashmir about 38-45 meters above the sea level. The plants were identified taxonomically and authenticated at the
Herbarium, Department of Botany, Kashmir university. Fresh leaves was washed thoroughly 2 - 3 times with running tap water and then with sterile water followed by shade-dried, powdered and used for extraction.

**Test microorganisms**

Human pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus* were collected from GMC medical college Bhopal India. Plant pathogenic bacteria such as *Xanthomonas vesicatoria* and *Ralstonia solanacearum* were collected from the culture collection of Department of Biotechnology, Barkatullah University Bhopal, India. All the test bacterial species were maintained on nutrient agar media.

**Preparation of aqueous plant extracts**

25 g of shade dried, powder of plant materials were macerated separately with 50 ml of sterile distilled water using pestle and mortar. The macerate was first filtered through four layer of muslin cloth and then filtrate was centrifuged at 8,000 rpm for 15 min at room temperature. Supernatant was filtered through Whatman filter paper and heat sterilized at 120ºC for 30 min. The extract was preserved aseptically in a brown bottle at 4oC until further use.

**Preparation of solvent extractions**

25 g of shade dried, powder of plant materials were filled separately in the thimble and extracted successively with 150 ml each of methanol, ethanol, ethyl acetate and chloroform using a Soxhlet extractor for 48 h. All the extracts were concentrated using rotary flash evaporator. After complete solvent evaporation, each of these solvent extract was weighed and preserved at 4oC in airtight bottles until further use. 1 g of each solvent residue was dissolved in 10 ml of respective solvents were used as the test extracts for antimicrobial activity assay.

**Anti-bacterial activity assay**

Antibacterial activity of aqueous extract and solvent extracts; methanol, ethanol, ethyl acetate and chloroform was determined by disc diffusion method on nutrient agar medium (Anonymous, 1996). Sterile Whatmann filter discs (6 mm diameter) were made in nutrient agar plate using sterile cork borer (5 mm) and inoculums containing 106 CFU/ml of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 50 μl each of all aqueous and solvent extracts were placed in the discs made in inoculated plates. The treatments also included 50 μl of solvents served as control and chloramphenicol as a standard control. The plates were incubated for 24 h at 37ºC and zone of inhibition if any around the wells were measured in mm (millimeter). Each treatment consists of three replicates and repeated at least twice. Minimum inhibitory concentration (MIC), was determined as the lowest concentration of *J. dolomiaea* plant extracts inhibiting the growth of the organism.

**RESULTS**

The ethnobotanical efficacy of various solvent extracts of *Jurinea dolomiaea* against both human and plant pathogenic bacteria showed varied level of inhibition (Table 1). Among treatments, maximum in vitro inhibition of tested bacteria *E. coli*, *S. aureus*, *X. vesicatoria* and *R. solanacearum* was scored in methanol extracts which offered inhibition zone of 10, 9, 12 and 12 mm respectively. Further, chloroform extract of *J. dolomiaea* was effective against all four tested bacteria which recorded significant inhibition zone of 12, 11, 7 and 9 mm respectively. Plant extract of *C. odorata* in methanol showed MIC of 2.0 mg/ml against phytopathogenic bacteria *X. vesicatoria* and *R. solanacearum*, whereas 4.0 mg/ml for clinical bacteria *E. coli* and *S. aureus*. Chloroform leaf extract showed MIC of 4.0 mg/ml against all tested bacteria. Aqueous extract showed MIC of 4.0 mg/ml against *E. coli* and *X. vesicatoria*, whereas MIC of 1.0 and 2.0 mg/ml were found against *S. aureus* and *R. solanacearum*, respectively (Table 2). The MIC of 2.0 and 4.0 mg/ml was found against all the tested bacteria when ethanolic extracts of *C. odorata* were used. The MIC of 4.0 mg/ml was found against *E. coli*, *X. vesicatoria* and *R. solanacearum* when ethyl acetate extract was used, whereas MIC of 1.0 mg/ml was found against *S. aureus*. 

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**Table 1**

<table>
<thead>
<tr>
<th>Plant Extract</th>
<th>MIC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td></td>
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<tr>
<td><em>S. aureus</em></td>
<td></td>
</tr>
<tr>
<td><em>X. vesicatoria</em></td>
<td></td>
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<tr>
<td><em>R. solanacearum</em></td>
<td></td>
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</tbody>
</table>

**Table 2**

<table>
<thead>
<tr>
<th>Plant Extract</th>
<th>MIC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td></td>
</tr>
<tr>
<td><em>X. vesicatoria</em></td>
<td></td>
</tr>
<tr>
<td><em>R. solanacearum</em></td>
<td></td>
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</tbody>
</table>
Plant based antimicrobial compounds have enormous therapeutical potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. The methanol, ethanol, ethylacetate and chloroform and aqueous extracts of the leaves of *J. dolomiaea* was subjected to a preliminary screening for antimicrobial activity against two human pathogenic bacteria *E. coli* and *S. aureus* and two phytopathogenic bacteria *X. vesicatoria* and *R. solanacearum*. It was clear from the present results, that both methanol and chloroform leaves extracts of *J. dolomiaea* exhibited pronounced activity against all the tested four bacteria. This tends to show that the active ingredients of the plant parts are better extracted with methanol than other solvents. The methanol extracts contain alkaloids, coumarins and tannins (Okemo, 1996). Coumarins and tannins have antibacterial and antihelminthic properties (Hedberg et al., 1983), also Eloff (1998) and Cowan (1999) found that methanol was more efficient than acetone in extracting phytochemicals from plant materials. The absence of antibacterial activity of chloroform, ethyl acetate and ethanolic extracts of *J. dolomiaea* indicates the insolubility of the active ingredients in these solvents. In general the activities against test bacterial culture used have shown good activity when compared with standard antibiotics. In another research, dichloromethane and aqueous extracts from the leaves as well as ethyl acetate extracts from the flowers have shown antibacterial activity against *Staphylococcus aureus* (Kameda et al., 1987). The minimum inhibitory concentration (MIC) for clinical bacteria was ranged between 0.35 to 4.0 mg/ml and 0.25 to 4.0 mg/ml for phytopathogenic bacteria when tested with all four solvents extracts of *J. dolomiaea*. Various investigators demonstrated that the extract of the leaves of *J. dolomiaea* at low concentrations (from 0.1 to 5 mg/ml) inhibits the growth of *Pseudomonas aeruginosa, E. coli, S. aureus* and *N. gonorrhoea* (Irobi, 1992; Bamba et al., 1993; Caceres et al., 1995). *Jurinea* species (Asteraceae) have been chemically investigated; flavonoids and terpenoids are extensively distributed in this genus (Amaro-Luis and Delgado, 1993; Biller et al., 1994). The presence of these flavonoids in *C. moritziana* could contribute to the observed antibacterial activity (Baez et al., 1998). In the present investigations the antibacterial activity of *J. dolomiaea* against phytopathogenic bacteria such as *Ralstonia solancearum* and *Xanthomonas vesicatoria* has been demonstrated for the first time. High activity against the Gram-positive organism *E. coli* was found in aqueous and all tested solvent extracts. Similar observations were

### Table 1. Zone of inhibitory activity (in millimeter) of different plant extracts against clinical and phytopathogenic bacteria.

<table>
<thead>
<tr>
<th>Source</th>
<th>Extract</th>
<th>E.coli</th>
<th>S. aureus</th>
<th>X. vesicatoria</th>
<th>R. solanacearum</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>J. dolomiaea</em></td>
<td>Aqueous</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
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<td>9mm</td>
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<tr>
<td></td>
<td>Ethanol</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td>Ethylacetate</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>12mm</td>
<td>11mm</td>
<td>7mm</td>
<td>9mm</td>
</tr>
</tbody>
</table>

### Table 2. Minimum inhibitory concentration (MIC) of *J. dolomiaea* for antibacterial activity.

<table>
<thead>
<tr>
<th>Source</th>
<th>Extract</th>
<th>MIC (mg/ml)</th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E.coli</td>
<td>S.aureus</td>
<td>X.vesicatoria</td>
<td>R.solanacearum</td>
</tr>
<tr>
<td><em>J.dolomiaea</em></td>
<td>Aqueous</td>
<td>4.00</td>
<td>1.00</td>
<td>4.00</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
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<td>4.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>2.00</td>
<td>4.00</td>
<td>2.00</td>
<td>4.00</td>
</tr>
<tr>
<td></td>
<td>Ethylacetate</td>
<td>4.00</td>
<td>1.00</td>
<td>4.00</td>
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</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol</td>
<td>8.00</td>
<td>10.00</td>
<td>9.00</td>
<td>8.00</td>
</tr>
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</table>
reported from nimbolide isolated from neem seed oil showing antibacterial activity against *S. aureus* and *Staphylococcus coagulase* (Nazma and Rao, 1977). Also antimicrobial effects of neem *e ccus mutans* and *S. faecalis* (Almas, 1999). These might be due to presence of triterpenoids, phenolic compounds, carotenoids, steroids, valainoids, ketones and tetratriterpenoids azadirachtin (Kraus, 1995). Shariff et al., 2006 reported that *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracted in chloroform were found to inhibit *E. coli* and *X. vesicatoria* at minimum inhibitory concentration (MIC) ranged between 0.25 to 6 mg/ml. *J.dolomiaea* methanolic extract posses a broad spectrum of activity against a panel bacteria responsible for the most common bacterial diseases. These primary extracts open the possibility of finding new clinically effective antibacterial compounds. *J.dolomiaea* providing active extract are found in different locations of Hemalia and are well known plants as most of them are used for various medical purposes (Phan et al., 2001). Continued further exploration of plant-derived antimicrobials is needed today. Further research is necessary to determine the identity of the antibacterial compounds from within plants and also to determine their full spectrum of efficacy. However the present study of in vitro antibacterial evaluation of plants forms a primary platform for further phytochemical and pharmacological studies to discover new antibiotic drugs.

REFERENCES


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