Comparative antimicrobial activities of different species of *Ixora*

Saleha Akter, Tahmina Haque, Esrath Jahan Irine, Md. Faisal Kabir, Shanta Ahmed, Taslima Begum

**Abstract**
Leaves of different plants of *Ixora* genus is traditionally used as poultice for various skin conditions and in the treatment of wounds to validate the traditional uses, the leaves of four species of *Ixora* such as *I. chinensis*, *I. lutea*, *I. coccinea* and *I. parviflora* were investigated to evaluate the in vitro antimicrobial activity by agar well diffusion method against nine bacterial strains of which three are gram positive bacteria (*Bacillus subtilis*, *Bacillus cereus* and *Staphylococcus aureus*) and six are gram negative bacteria (*Escherichia coli*, *Acinetobacter*, *Salmonella paratyphi*, *Salmonella typhi*, *Klebsiella*, *Proteus* and *Pseudomonas*). The methanol extracts of the leaves of *I. chinensis*, *I. lutea* and *I. coccinea* did not show any antimicrobial activity, whereas the extract of *I. parviflora* showed significant antimicrobial activity against *Salmonella paratyphi* (19 mm), *Bacillus subtilis* (18 mm), *Salmonella typhi* (16 mm) and *Acinetobacter* (13 mm). Gentamicin (10 µg/disc) was used as standard drug. The results of the present study find only *I. parviflora* effective against some organisms.

**Keywords:** Antimicrobial, Agar well diffusion method, Zone of Inhibition, Ixora.

1. **Introduction**
Medicinal plants have been used as a rich source of antimicrobial agents in different regions of the world from ancient time [1, 2]. Various plant parts and their extracts possess varied medicinal properties due to the presence of a wide range of chemical constituents in them. The potential of plants as a source of drugs is still to be vastly explored. Multiple drug resistance has become a critical problem in pharmacotherapeutics as there are increasing numbers of diseases exhibiting various levels of drug resistance, including bacterial infections [1]. Herbal medications and phytochemical screening of various plant species for medicinal leads are now receiving much attention. Some of the herbal medicines that are being considered as a source of new antibacterial drugs have been time-tested for thousands of years and are comparatively safe for both human use and the environment [3].

*Ixora* is a genus of the flowering plants from the family Rubiaceae containing around 500 species where names of the plants differ on the basis of colours of the flowers. *Ixora* plant and its various parts like flowers, leaves, roots, barks and fruits have been used by various ethnic groups of Africa, Asia and Europe [4]. In Bangladesh it is cultivated throughout the country as a garden plant for beautiful flowers. The leaves of these plants are reported to have spasmyloytic, antiinflammatory, anti-diarrheal, antiasthmatic, hypotensive, antilucre, spasmolytic, antiviral, antigestagenic and anticancer activity [5]. Leaves are also used in skin diseases, colic, flatulence, diarrhea, indigestion, ulcers, wounds, headache and stomachache. Flowers are used for amenorrhea, hypotension, whooping cough, ulcers, dysentery, haemoptysis and cancer. They also possess estrogenic and abortifacient properties [6, 7, 8]. The root of this plant is used in skin diseases, amenorrhea, leucorrhea, bronchial disorders and urinary trouble. They are also used as astringent and antiseptic [6, 8]. Barks are used as decoction for the ailment of anemia [3]. Various pharmacological studies on this plant have revealed the antimicrobial [9], antiasthmatic [10], antiviral, antitumor [11], spasmyloytic, hepatoprotective [12], antioxidant [13], anthelmintic [14], antidiarrhoeal [15], antinociceptive [16], antiinflammatory [17], hypoglycemic and hypolipidemic [8], antilucre [18], cytotoxic and antitumor [11], antiancancer [19], antidiarrhoeal [20] and hypotensive activities [9]. Due to the wide spread use of various parts of this plant in traditional system of medicine, the chemical analysis of various species has been done and revealed the presence of various phytoconstituents like lupeol, linolic acid, urosolic acid, stearic acid and sitosterol [4]. Flowers are reported to contain leucocyanidin glycoside, rutin; root bark contains octadecadienoic acid,
leaves contain epicatechin, ixoratin A-2, procyanidin A2 [21]; seeds contain ixoric acid, palmitic acid, stearic acid, oleic acid, and crepenynic acid [9]. To evaluate the potentiality of the leaves of Ixora plants as antimicrobial agent and to find any possible correlation of their use in skin diseases we have studied the antimicrobial activities of the leaves of four different species of Ixora plant.

2. Plant material and preparation of Extract
The fresh leaves of I. chinensis, I. lutea, I. coccinea and I. parviflora were collected in August 2013 from Ramna Park, Dhaka, Bangladesh. The leaves were washed and then dried. The dried leaves were grinded and made into coarse powder. 100 gm powder of each was then macerated separately with 400 ml methanol with occasional stirring. Then the extracts were filtered and dried. The crude extracts were then used for the study.

3. Microorganisms
Pure cultures of three gram positive bacteria such as Bacillus subtilis, Bacillus cereus and Staphylococcus aureus and six gram negative bacteria such as Escherichia coli, Acinetobacter, Salmonella paratyphi, Salmonella typhi, Klebsiella, Proteus and Pseudomonas were collected from the Microbiology Laboratory, Department of Pharmacy, Primeasia University, Banani, Dhaka, Bangladesh in the month of October 2013.

4. Antimicrobial activity by Agar well diffusion method
Bacterial suspensions were uniformly spread over sterile agar petri dishes with the help of sterile cotton swab. The plates were then kept for 5 min. Then wells of 7 mm in diameter was punched into all of the agar petri dishes and filled with 30 μl (0.24 mg/ml) methanol extracts of leaves of I. chinensis, I. lutea, I. coccinea and I. parviflora in each plate. The plates were then allowed to diffuse for 1 hr at room temperature. The plates were then incubated at 37 ºC for 24 hrs. After incubation confluent growth of bacteria was observed and the inhibition zone of bacterial growth was measured in mm by measuring the scale [22]. Gentamicin (10 μg/disc) was used as standard. The antimicrobial activity was calculated as the difference between the diameter of inhibition zone of bacterial growth was measured in mm by using the diameters of wells with and without the extracts. The averages were taken as the result.

5. Results and Discussion
The antimicrobial activities of the leaf extracts of I. chinensis, I. lutea, I. coccinea and I. parviflora were studied and the result of the study was given in the table. These extracts were tested against nine bacterial strains and three of the extracts such as I. chinensis, I. lutea, I. coccinea were found to possess no antimicrobial activity against any of the bacteria except the extracts of I. parviflora. The extract of the leaves of I. parviflora were found to possess strong antimicrobial activity against Bacillus subtilis (18 mm), Salmonella paratyphi (19 mm), Salmonella typhi (16 mm), and Acinetobacter (13 mm) while rest of the organisms were not sensitive to this extract and did not show any zone of inhibition. Gentamicin (10 μg/disc) was used as standard in this experiment and showed significant antimicrobial activity against all the tested bacteria. In case of Bacillus subtilis the extract showed a similar inhibition pattern with the standard and in the case of Salmonella paratyphi the extract inhibited more zone than the standard though no activity against the other organisms.

Previous results from the reports on the antimicrobial activity of different species of Ixora species can be compared to the present study as there are some differences in the result. Previous reports showed that the leaves of various species of Ixora possess significant antimicrobial activity [9, 19, 23] and in our present work the leaves of I. chinensis, I. lutea, I. coccinea showed no antimicrobial activity while the leaves of I. parviflora showed significant antimicrobial activity. The differences between these results might be due to the differences in the concentration of the extracts used for the experiment. It can also be stated that as the extract of I. parviflora showed strong antimicrobial activity, even with the concentration of 0.24 mg/ml, this plant is supposed to have some important chemical constituents responsible to exert an antimicrobial effect. Plant secondary metabolites are scientifically proved chemical constituents responsible for exerting antimicrobial properties of plant extracts. Previous reports on the phytochemical screening of this plant have found the presence of flavonoids, tannin, saponins, aromatic oil, fatty acid and a lot of other phytoconstituents in them [4] so it can be correlated with the present study.

Due to the lack of thorough clinical studies and limited toxicological data available, the safe and effective use of medicinal plant is difficult [24]. Ixora plants are very known and trusted medicinal plants in the traditional system of medicine. Further evaluation of other parts of this plant for antimicrobial activity with in vivo evaluation and purification of the active compounds with support of toxicity studies of various extracts from Ixora species are suggested.

6. Conclusion
The study shows that leaves of I. parviflora have good antimicrobial activity against four microorganisms (Bacillus subtilis, Acinetobacter, Salmonella paratyphi and Salmonella typhi).

Table: Result of antimicrobial activity of different species of Ixora plant by Agar well diffusion method.

<table>
<thead>
<tr>
<th>Test Microorganisms</th>
<th>Gentamicin (10 μg/disc)</th>
<th>Ixora lutea 30 μl</th>
<th>Ixora chinensis 30 μl</th>
<th>Ixora coccinea 30 μl</th>
<th>Ixora parviflora 30 μl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter</td>
<td>17</td>
<td>--</td>
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<td>--</td>
<td>13</td>
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<tr>
<td>Bacillus subtilis</td>
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<td>18</td>
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<td>Salmonella typhi</td>
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<td>16</td>
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<td>Proteus</td>
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7. References


