Search for an herbal medicine: Antibacterial activity of methanolic extract of *Haldina cordifolia*

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**Abstract**

*In-vitro* antibacterial activities of methanolic extract of *Haldina cordifolia* were evaluated against a series of various bacterial strains by well diffusion method using nutrient agar medium. Antibacterial activities were found to be quite inspiring as it has shown inhibitory effect against all the pathogens like *Staphylococcus aureus* ATCC 29757, *Pseudomonas aeruginosa* ATCC 25619, *Escherichia coli* ATCC 10536, *Streptococcus pneumoniae*, *Clostridium difficile*, *Klebsiella pneumonia*, *Mycobacterium tuberculosis* and *Bacillus subtilis* ATCC 10389. Antibacterial activity was determined by the well diffusion method using nutrient agar medium. Tetracycline was used as a positive control. The *Haldina* extract restricted alkaloids, flavonoids and fixed oils.

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**Key words:** *Haldina cordifolia*, Herbal medicine, Antibacterial activity.

1. Introduction

Herbalism or "herbal medicines" have a long history to cure several kinds of human diseases from the various parts of the plants such as leaf, stem, bark, root, etc [1]. Plants have been the basis for medical treatments through much of human history, and such traditional medicine is still widely practiced today. Modern medicine recognizes herbalism as a form of alternative medicine, as the practice of herbalism is not strictly based on evidence gathered using the scientific method. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis, and quinine. The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicines for some aspect of primary health care. The use of, and search for, drugs and dietary supplements derived from plants have accelerated in recent years. Pharmacologists, microbiologists, botanists, and natural-products chemists are combining the Earth for phytochemicals and leads that could be developed for treatment of various diseases. Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80 percent show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived. More than two thirds of the world's plant species at least 35,000 of which are estimated to have medicinal value come from the developing countries [1].

It was realized from the various reports that *Haldina cardifolia*, belongs to the family Rubiaceae, have displayed plethoras of potential biological activities such as anticancer [2], antulcer [3], hepatoprotective [4], anti-inflammatory [5], antifertility [6], antidiabetic [7], antiamoebic [8], antinociceptive [9] etc. from its various parts. Furthermore, this plant have also used in curing various ailments such as rheumatism [10], stomachache[11], headache [12], cold/cough [13], toothache [14], fever [15], pain and swelling [16], bacterial infection [17], urinary problems [18], conjunctivitis [19], miscarriage [20] etc. Keeping the view of the above mentioned medicinal importance of this plant, we therefore became interested to investigate the antibacterial activity of this plant. To the best of our knowledge, the antibacterial activity from this plant has not been reported so far till now. In the present paper, we would like to explore the antibacterial activity of
the methanolic extract this plant employing various kinds of strains. It was found that majority of these strains employed, have displayed potential antibacterial activity.

1.1 Introduction to the plant:

**Family:** Rubiaceae  
**Vernacular names:**  
**English:** Yellow teak  
**Sanskrit:** Haridru  
**Hindi:** Haldu  
**Telugu:** Pasupukadamba  
**Kannada:** Hethega, Arisina tega  
**Adina cordifolia** Roxb. (Synonym: *Haldina cordifolia*)

1.2 Morphology

A hulking deciduous tree with the shoot often irregularly fluted and buttress at the bottom and normally reaching 6m girth and 15-35m in height. It is a blossoming plant in the family Rubiaceae. Bark to a degree grey, thick, bumpy or blackish and to a certain extent pale ashy or silvery white, wood yellow moderately rigid and even-grained. Leaves opposite 10-25cm width, orbicular, abruptly acuminate, pedestal cordate, glabrous above pubescent beneath, with 5-8 pairs of sideways nerves. Petiole 3.8-10cm extended stout, juvenile. Stipules 1.3-1.8cm long oblong or obovate. Flowers are irrelevant individually but are very attractive when they blossom together in balls with a perimeter of 2 to 3cm. They are usually yellow in shade often tinged with a shade of pink. Fruits capsules split into 8 pairs of sideways nerves. Petiole 3.8-10cm extended stout, juvenile. Stipules 1.3-1.8cm long oblong or obovate. Flowers are irrelevant individually but are very attractive when they blossom together in balls with a perimeter of 2 to 3cm. They are usually yellow in shade often tinged with a shade of pink. Fruits capsules split into 8 pairs of sideways nerves. Petiole 3.8-10cm extended stout, juvenile. Stipules 1.3-1.8cm long oblong or obovate. Flowers are irrelevant individually but are very attractive when they blossom together in balls with a perimeter of 2 to 3cm. They are usually yellow in shade often tinged with a shade of pink. Fruits capsules split into 8 pairs of sideways nerves.

2. Material and Methods:

2.1. Plant material

The plant material used in this learning is the dried leaf of plant *Haldina cordifolia* which is collected from the Puri district of Orissa state of India, Was authenticated in the Department of Botany, Utkal University, Bhubaneswar. Fresh leaves were washed in running water, shade dried and crushed to fine powder.

2.2. Preparation of extract:

The powdered leaves were defatted using petroleum ether using Soxhlet apparatus for 48 hrs. The resulted marc was further extracted with methanol by Soxhlet apparatus for 72 hrs. The extract was then filtered and evaporated to dryness in vacuum. The yield of Methanolic extract was calculated and it was found to 4.5 %. The marc left behind was kept in refrigerator for further use.

2.3. Tested Microorganisms

Different strains were used in this study: methicillin resistant *Staphylococcus aureus* ATCC 29757, multi drug resistant *Pseudomonas aeruginosa* ATCC 25619 (i.e. resistant to cefuroxime, cefotaxime, gentamycin, amikacin, erythromycin, clindamycin, ofloxacin, nalidixic acid, norfloxacin, ciprofloxacin, amoxicillin-clavulanic acid, and then enterohemorrhagic *Escherichia coli* ATCC 10536, penicillin and other beta-lactams resistant *Streptococcus pneumoniae*. Clindamycin-resistant *Clostridium difficile* is a nosocomial pathogen that causes diarrheal disease, Highly drug-resistant Gram-negative bacilli *Klebsiella pneumonia*, Multi-drug-resistant tuberculosis bacteria *Mycobacterium tuberculosis* and a reference strain *Bacillus subtilis* ATCC 10389 was also tested.

2.4. Phytochemical screening

The Haldina extract restricted alkaloids, flavonoids, fixed oils but no glycosides and tannins were detected. The alkaloids were established in both chloroform and methanol extract, whereas flavonoids segment was bring into being in methanol extract. Fixed oil is seen in petroleum ether extract.

2.5. Antibacterial activity

Antibacterial activity was determined by the well diffusion method using nutrient agar medium, 100μl of suspension containing 10⁶ colony forming units μl⁻¹ of bacteria spread over the nutrient agar medium plates using separate sterile cotton buds. After the microbial lawn preparation two different concentration of extract were placed into each well petridishes were incubated at 37°C for 24 hours and the average diameter of the inhibition zone surrounding the wells were determined visually. The different concentration used are 25μg in 10μl DMSO (25μg in 10μl) and 50mg in 10ml DMSO (50μg in 10μl). A standard of 30μg tetracycline disk was used as an positive control.

3. Results and Discussion

The antibacterial activity of extract obtained from the plant under the study by the diffusion method are mentioned in Table 1 and different observations after antibacterial activity a-h is shown in Figure 2. The methanolic extract of the plant haldina cordifolia showed the inhibitory effect in (25μg in 10μl and 50μg in 10μl) inhibition zone against...
Table 1: Antibacterial activity employing different strains

<table>
<thead>
<tr>
<th>Micro organism</th>
<th>Standard drug 30μg/10μl</th>
<th>Methanolic extract 25μg/10μl</th>
<th>Methanolic extract 50μg/10μl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>+</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>+</td>
<td>++</td>
<td>++</td>
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<tr>
<td>Pseudomonas aeruginosa</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Clostridium difficile</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

Figure 2: Different observations after antibacterial activity a-h.

bacillus subtilis, Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae, Clostridium difficile, Klebsiella pneumonia, Mycobacterium tuberculosis and pseudomonas aeruginosa. The maximum effect was shown on the Bacillus subtilis, Streptococcus pneumoniae, Clostridium difficile, Klebsiella pneumonia, and Escherichia coli, whereas higher doses required for the pseudomonas aeruginosa, Mycobacterium tuberculosis and staphylococcus aureus.

Antibacterial activity of the extract were expressed by (-) no zone of inhibition (+) zone of inhibition = 8mm in diameter (++) zone of inhibition > 8mm in diameter. All the tests were performed in duplicate and repeated for conformation of result.
4. Conclusions
The result indicated the crude extract of the corresponding plant species studied showed antibacterial activities towards gram positive bacteria (Bacillus subtilis) and multi drug resistant Staphylococci aureus. The resistance of gram negative bacteria enterohemorrhagic Escherichia coli and pseudomonas aeruginosa to plant extract was not unexpected as in general, this class of bacteria is more resistant than gram positive bacteria. Such resistance could be due to the permeability barrier provided by the cell wall of the membrane accumulation mechanism [21]. Bilefection caused by pseudomonas aeruginosa especially those of multi drug resistance are among the most difficult to treat with conventional antibiotics. In our study the growth of P. aeruginosa was remarkably inhibited by the methanolic extract of Haldina cordifolia. It seems very likely the antibacterial compound extracted from Haldina cordifolia may inhibit bacteria a different mechanism than that of currently used antibiotics and may have therapeutic values as an antibacterial agent against multi drug resistant bacterial strains.

Enterohemorrhagic Escherichia coli are increasingly isolated from severe diarrhea disease and constitute a serious medical problem for many patients. Infection with these organism may result in life threatening complication such as hemolytic anemia syndrome (HUS) and thrombotic thrombocytopenic purpura. Our results demonstrate that the methanolic extract of Haldina cordifolia displayed antimicrobial activity against Enterohemorrhagic Escherichia coli 25 microgram/10 micro ml inhibition zone. The plant may thus be a source that could be useful in the treatment of infection caused by this type of organism. Numerous studies have been carried out to extract various natural products for screening of antibacterial activity but in our study the plant Haldina cordifolia is showing the broad spectrum activity as it is inhibiting two important gram positive and two gram negative bacteria.

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