Antimicrobial Activity of Aristolochia tagala Cham. Centella asiatica Linn. Houttuynia cordata Thunb. on Multi-Drug Resistant Clinical Isolates.

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ABSTRACT

The objective of this study is to evaluation of antimicrobial activity of Aristolochia tagala Cham. (AT), Centella asiatica Linn. (CA), Houttuynia cordata Thunb (HC) on multi-drug resistant bacteria isolated from clinical samples. Alcoholic extracts of these plants carried out by adopting Kirby-Bauer disc diffusion method against standard strains (ATCC) and resistant clinical isolates of Staphylococcus aureus, Enterococcus faecalis and Staphylococcus aureus. Piperacillin, ampicillin, amikacin, ciprofloxacin, and cefotaxime served as positive controls in the standard test and nitrofurantoin, teicoplanin, linezolid, and vancomycin were used in the testing of the resistant isolates. 100 mg/mL of the plant extracts were used as the test extracts taking 2.5 % DMSO as a negative control. From the zone of inhibition (mm) recorded, AT demonstrated higher antimicrobial efficacy against Enterococcus and S. aureus. Another significant finding is HC showed considerable effectiveness against E.Coli. However, methanolic extract of CA showed only moderate antimicrobial activity. Good antibacterial activity was seen with AT alcoholic extracts for Enterococcus and S. aureus resistant strains in clinical isolates. Antibacterial activity of HC against E. coli isolates also holds promising results.

Keywords: Zone of inhibition, phytochemical screening, disc diffusion method, resistant clinical isolates

INTRODUCTION

Medicinal plants have cornered attention in the treatment of various disorders and ailments recently although their use has already been reported for thousands of years through traditional and folk practices. The immense potential of these plants as an alternative solution to health problems has been studied thoroughly especially in modern era of antimicrobial resistance.¹ The World Health Organization (WHO) reported that almost 80% of the world’s population is depending on traditional medicine and traditional treatment.² Centella asiatica L. which belongs to the family Mackinlayaceae is an herbal plant that has been used since ancient times. It is commonly known as Brahmi or Mandooparni. According to the Ayurvedic system of medicine, it has been used as a brain tonic and in the treatment of various chronic diseases and mental disorders. Pharmacological activities like wound healing, antibacterial, antioxidant and anticancer have also been reported.³ The chemical constituents present in it includes asiaticoside, madecassoside, acids like asiatic acid, made cassic acid, sugars like glucose, rhamnose, plant sterols like sitosterol, stigmasterol and also vitamins like ascorbic acid. CA has also been useful in treating inflammations, diarrhea, asthma, tuberculosis and a variety of skin disorders like leprosy, lupus, psoriasis, and keloid.⁴ Aristolochia tagala Cham. is also known as Indian birthwort or Dutchman’s pipe. The chemical constituents present in the roots of this plant are aristolochic acid, allantoin, alkaloid aristolodin, essential oils, sesquiterpene hydrocarbon, ishwarane and an alcohol ishwararol, aristolactam illa, beta sitosterol, kempferol, stigmasterol, caffeoylquinic acid.⁵ Aristolochia tagala has been reported to have been used as an antidote for cobra poison as it destroys the toxic effects of all poisons and acts as a blood purifier. The antifertility activity has also been seen from its inhibitory activity on estrogen production. Antimicrobial activity of the ethanolic extract of its root has been studied in strains of Staphylococcus aureus.⁶ Other pharmacological effects include cytotoxic, antiproliferative, antifungal, analgesic and antibacterial activities. Houttuynia cordata is a perennial plant native to mountainous regions of eastern Asia and belongs to the Saururaceae family. Anti viral properties have been studied in this plant and it showed to be effective against important viruses such as Herpes Simplex Virus-1 (HSV-1), influenza virus, and Human Immunodeficiency Virus-1 in vitro.⁷ It is widely used in...
Korea as a medicinal herb for the treatment of various conditions; some of them were cough, pneumonia, bronchitis, dysentery, acne and nasal polyps. Its use in stimulating the immune system and as an anticancer agent has also been reported. The antimicrobial activity and its use in the control of infection, as well as an anticancer, have been studied in Japan. The indigenous tribe of Arunachal Pradesh, India used this plant in the treatment of heart disorders and as a sedative. Apart from its medicinal uses, *Houttuynia cordata* has also been reported. The purpose of this study is to find out if the plant extracts of *Aristolochia tagala* Cham., *Centella asiatica* Linn., and *Houttuynia cordata* Thunb. shows the desired antimicrobial activity against the resistant clinical isolates of interest.

**MATERIALS AND METHODS**

**Plant materials**

*A. tagala* was collected as a whole plant from Ri-Bhoi District of Meghalaya, India. The plant was identified by Dr. R. Gogoi, Botanical Survey of India, Arunachal Pradesh Regional Centre, Itanagar, India by comparing the voucher specimen 18721, Botanical Survey of India, Shillong. Roots of *A. tagala* were air-dried and used. *C. asiatica* and *H. cordata* were collected locally and identified by Dr. P. B. Gurung, Curator, Department of Botany, North-Eastern Hill University (NEHU), Shillong with voucher specimen (No. AKY/003). The leaves were air-dried, powdered and used for extraction purposes.

**Reagents**

Methanol and ethanol for extraction purposes; solvent Dimethylsulphoxide (DMSO) and hexane were procured from S.D. Fine Chem Ltd., India.

**Extraction procedure and preparation of plant extracts**

The powdered dried leaves of *C. asiatica* and *H. cordata* were defatted three times before undergoing the extraction process with three volumes of hexane and ethanol respectively. Each 30g of powdered *A. tagala* and *C. asiatica* was mixed separately with 150 mL of 80% methanol and 30 g of powdered *H. cordata* was mixed with 150 mL of 80 % ethanol in three respective round bottom flasks, closed by foil paper and placed on a shaker at 37 °C for 48 hrs. The crude extract was then filtered by paper and placed on a shaker at 37 °C for 48 hrs. The final yield was 5 g/kg of dried roots of *Aristolochia tagala*, 12.87 g/kg of dried leaves of *Centella asiatica* and 15.6 g/kg of dried leaves of *Houttuynia cordata*.

**Phytochemical screening**

The phytochemical screening for the presence of phenols, tannins, flavonoids, and steroids was carried out accordingly. The purpose of this study is to find out if the plant extracts of *Aristolochia tagala* Cham., *Centella asiatica* Linn., and *Houttuynia cordata* Thunb. shows the desired antimicrobial activity against the resistant clinical isolates of interest.

**Sensitivity test on Standard Strains**

The antibacterial screening of our plant extracts was carried out using the well diffusion method against four standard bacterial strains of *Escherichia coli* (ATCC-25922), *Enterococcus faecalis* (ATCC-29212) and *Staphylococcus aureus* (ATCC-25923). 100 mg/ml of the plant extracts dissolved in 2.5% of DMSO was used as the test extracts, taking 2.5 % DMSO as a negative control. Antibiotics wells of piperacillin, ampicillin, amikacin, ciprofloxacin, and cefotaxime served as a positive control. The zone of inhibition (mm) against the selected pathogens was determined and recorded.

**Sensitivity test on Isolated Strains**

Multidrug-resistant isolates collected from patients’ urine, sputum and stool were taken and the antimicrobial activity of the extracts of *A. tagala*, *C. asiatica*, and *H. cordata*. on these resistant isolates were tested. 100 mg/ml of the plant extracts dissolve in 2.5% of DMSO was used as the test extracts for antimicrobial activity assay taking 2.5 % DMSO as a negative control and compared with standard antibiotics like nitrofurantoin, teicoplanin, linezolid, and vancomycin. The zone of inhibition against the selected pathogens (mm) was determined and recorded.
Sample collection: All clinical samples were collected from patients’ urine, sputum and stool samples and screened for antimicrobial resistance in the Department of Microbiology, NEIGRIHMS. The resistant isolates were then taken for the study in which 20 repetitive resistant isolates of each of the strain (n=20) were tested subsequently.

Statistical analysis

The antimicrobial activity was measured as zone of inhibition (mm) expressed as mean (SD). Median and range was also calculated. The number of clinical isolates taken was 20 (n=20).

RESULTS

Phytochemical screening

After performing all the tests for the presence of phytochemicals such as phenols and tannins, flavonoids and steroids, it was found that all the three plant extracts namely, the methanolic extract of Aristolochia tagala, methanolic extract of Centella asiatica and ethanolic extract of Houttuynia cordata showed the presence of these phytochemicals. This was confirmed by the respective color changes that were noted in the test extracts during the test process. The results are represented in Table 1.

Table 1: Identification tests for the presence of phenols and tannins, flavonoids and steroids.

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Phenols and tannins</th>
<th>Flavonoids</th>
<th>Steroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>CA</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>HC</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

AT= Aristolochia tagala; CA = Centella asiatica; HC= Houttuynia cordata; +++= present.

Antimicrobial activity

Sensitivity test on Standard Strains: The diameter of the zone of inhibition (mm) of all the three extracts on the standard strains of Enterococcus, E. coli and Staphylococcus aureus are shown in Table 2.

Table 2: Diameter of zone of inhibition of different plant extracts and standard antibiotics tested against standard bacteria strains (ATCC) of Enterococcus, E. coli and Staphylococcus aureus

<table>
<thead>
<tr>
<th>Strain</th>
<th>Diameter of zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AT</td>
</tr>
<tr>
<td>Enterococcus ATCC-29212</td>
<td>31 mm</td>
</tr>
<tr>
<td>E. coli ATCC-25922</td>
<td>14 mm</td>
</tr>
<tr>
<td>S. aureus ATCC-25923</td>
<td>20 mm</td>
</tr>
</tbody>
</table>

AT= Aristolochia tagala; CA = Centella asiatica; HC= Houttuynia cordata

Table 3: Mean, Standard Deviation, Median, and Range of zone diameters (mm) of the plant extracts against clinical isolates of Enterococcus susceptible to Nitrofurantoin, Teicoplanin, Linezolid and Vancomycin

<table>
<thead>
<tr>
<th>Enterococcus (n=20)</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AT</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>21.15(2.66)</td>
</tr>
<tr>
<td>Median</td>
<td>20.5</td>
</tr>
<tr>
<td>Range</td>
<td>10</td>
</tr>
</tbody>
</table>

AT= Aristolochia tagala; CA = Centella asiatica; HC= Houttuynia cordata; Nitro=Nitrofurantoin, Teico=Teicoplanin, Linez=Linezolid, Vanco= Vancomycin

Sensitivity test on Isolated Strains: From the results of the well plate method, it was observed that AT showed a significant difference as compared to CA and HC against clinical isolates of Enterococcus demonstrating a higher antimicrobial efficacy as compared to the other two extracts. AT also showed a significant antimicrobial activity against the isolates of Staphylococcus as compared to CA and HC. But the same antimicrobial efficacy was not seen in case of its activity against E.Coli. The ethanolic extract of HC showed a considerable effectiveness against E.Coli when compared to the activity of the other two extracts. Although, it is to be noted that HC was found to be ineffective against the strains of Enterococcus and S. aureus. On the other hand, the methanolic extract of CA showed no difference in its activity against any of the clinical bacterial strains tested as compared to the methanolic and ethanolic extract of AT and HC respectively. The results of the antimicrobial sensitivity test are shown on Table 3, 4 and 5.
Table 4: Mean, Standard Deviation, Median, and Range of zone diameters (mm) of the plant extracts against clinical isolates of E.coli susceptible to Nitrofurantoin and Teicopalin

<table>
<thead>
<tr>
<th></th>
<th>AT</th>
<th>CA</th>
<th>HC</th>
<th>Nitro</th>
<th>Teico</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>12.30 (1.14)</td>
<td>11.85 (1.06)</td>
<td>20.45 (1.46)</td>
<td>22.05 (1.85)</td>
<td>11.10 (0.94)</td>
</tr>
<tr>
<td>Median</td>
<td>12</td>
<td>12</td>
<td>20</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>Range</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

AT= Aristolochia tagala; CA = Centella asiatica; HC= Houttuynia cordata; Nitro=Nitrofurantoin, Teico=Teicopalin

Table 5: Mean, Standard Deviation, Median, and Range of zone diameters (mm) for the plant extracts against clinical isolates of S. aureus susceptible to Nitrofurantoin, Linezolid and Vancomycin

<table>
<thead>
<tr>
<th></th>
<th>AT</th>
<th>CA</th>
<th>HC</th>
<th>Nitro</th>
<th>Linez</th>
<th>Vanco</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>22.25 (2.27)</td>
<td>12.05 (1.24)</td>
<td>13.6 (1.35)</td>
<td>13.35 (1.38)</td>
<td>23.15 (1.52)</td>
<td>23.05 (1.77)</td>
</tr>
<tr>
<td>Median</td>
<td>22</td>
<td>12</td>
<td>14</td>
<td>13.5</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Range</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

AT= Aristolochia tagala; CA = Centella asiatica; HC= Houttuynia cordata; Nitro=Nitrofurantoin, Linez=Linezolid, Vanco=Vancomycin

**DISCUSSION**

The presence of complex naturally occurring phytochemicals in plants and other natural sources have been shown to be beneficial and important sources of new drug entities. These secondary plant metabolites proved to be effective and potential antimicrobial agents. The phenolic compounds found in plants have been known to have antimicrobial characteristics. The polyphenols along with sterols have been found to inhibit bacteria and fungi growth. Flavonoids, on the other hand, have been studied for its antimicrobial activity and were found that, higher the flavonoid content, higher is the antimicrobial effect. These secondary metabolites have been found to exhibit wide pharmacological activities like anticancer, antiviral, antimutagenic and anti-inflammatory activities. According to Cushnie et al., further investigations into the mechanism of action as well as their interaction between these active flavonoids and their target sites might lead to the development of novel drug molecules having potential antimicrobial activity.

The presence of steroidal components has also been found to elucidate low antimicrobial activity against S. aureus, E. coli, S. Typhimurium and C. Albican. Another study also showed that steroidal compounds, namely corticosterone, and beta-sitosterol, showed inhibition of the growth of microorganisms like P. multocida and S. aureus respectively. The plant extracts of A. tagala, C.Asiatica and H.Cordata showed the presence of these active phytochemicals, hence the presence of these secondary plant products might help in the elucidation of their respective antimicrobial activities.

Species of A.tagala has traditional uses as an anticancer, antifungal, antibacterial and anti-infective agent. Antimicrobial activity of the ethanolic extract of A.tagala roots has been reported in strains of Staphylococcus aureus. In our study, the inhibitory action was seen to be effective in strains of Staphylococcus, Enterococcus and E. coli. The plant extracts of H. cordata has been reported to possess inhibitory actions against microorganisms like S. aureus and S. ureae as well as a potential treatment option against Salmonella, Brucella, Listeria, Bordetella and Helicobacter. Its antimicrobial activity against S. aureus was further reported in a study that documented the ability of H. cordata to inhibit strains of E. coli as well as moderate bacteria inhibition activity against S. aureus which is similar to our study showing median of (20 mm for E. coli and 14mm for S. aureus). Traditionally, Casiatica has been used as an antioxidant and antibacterial agent. Casiatica root and leaf extracts were reported to have antimicrobial activity in many bacterial isolates like E. coli, Staphylococcus aureus, Aspergillus flavus, Pseudomonas and Candida albicans. From our study, however, the leaf extract of CA exhibit a small significant antimicrobial activity against all the bacterial isolates namely, Enterococcus, E. coli and Staphylococcus. Roots of CA have also been studied and reported that it has more potential inhibitory action as compared to its leaves.

**CONCLUSION**

The antibacterial activity have been seen with methanolic extract of Aristolochia tagala against resistant strains of clinical isolates of Enterococcus and S. aureus. Ethanolic extract of Houttuynia cordata have good antimicrobial activity against resistant E. coli isolates. These activities holds promising results for the exploitation of antimicrobial activity of these natural occurring plants which are easily available and can serve as potential new
drug entities for combating the ever-rising antimicrobial resistance seen with already available antibiotics.

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REFERENCES


20. Idris FN, Nadzir MM. Antimicrobial activity of Centella asiatica on Aspergillus niger and Bacillus subtilis.
Chemical Engineering Transactions. 56, 2017 Mar 20, 1381-6.


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Conflict of Interest: None declared.

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