Research Article

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 *Staphyllococcus aureus*, *Enterococcus faecalis* and *Staphyllococcus 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

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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 Mandookparni. 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, madecassoide, 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 IIIa, beta sistosterol, 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 *Staphyllococcus aureus*.[6] other pharmacological effects include cytotoxic, antiproliferative, antifungal, analgesic and antibacterial activities. *Houttuynia cordata* Thumb. Is a perennial plant native to mountainous regions of eastern Asia and belongs to the *Saururaceae* family. Antiviral 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 been used as cosmetics in preventing or treating wrinkles, antiaging, skin whitening and improving skin conditions owing to its anti-inflammatory and skin calming effect. Inhibitory effect against strains of *Staphyllococcus* aureus has also been reported.[8] 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.[9, 10] 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 passing the extract through Whatman No. 1 filter paper and then concentrated. After complete solvent evaporation, extracts were weighed and stored in a refrigerator at 4 °C for further use. 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.[11]

Test for the presence of phenols and tannins

An approximate amount of crude extract was mixed with 2 mL of 2% solution of $FeCl_3$. Black coloration indicated the presence of phenols and tannins.

Test for the presence of flavonoids

Shinoda test

The crude extract was dissolved in methanol and few fragments of magnesium ribbon were added and mixed. Concentrated hydrochloric acid was added dropwise. Pink reddish/scarlet color indicated the presence of flavonoids.

Test for the presence of steroids

The crude extract was mixed with 2 mL of chloroform and concentrated sulphuric acid was added from the sides of the test tube. A red color was formed in the lower chloroform layer indicated the presence of steroids.

Antimicrobial activity

Antibiotic susceptibility test was conducted by adopting the Kirby-Bauer disc diffusion method.[12] Antimicrobial susceptibility testing was performed on Mueller Hinton agar. The cultures were streaked closely with a swab on the medium in the form of lawn and well diameter was made of 10 mm size. Plates were incubated at 37^o C overnight.

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 *Staphyllococcus* 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 dissolved 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.

Plant extracts	Phenols and tannins	Flavonoids	Steroids
AT	+++	+++	+++
CA	+++	+++	+++
HC	+++	+++	+++

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 *Staphyllococcus 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 *Staphyllococcus aureus*

Strain	Diameter of zone of inhibition							
	AT	CA	HC	Amp	Ami	Cipro	Cefo	Pipera
***Enterococcus* ATCC-29212**	31 mm	14 mm	14 mm	25 mm	14 mm	27 mm	14 mm	-
***E.coli* ATCC-25922**	14 mm	12 mm	19 mm	20 mm	20 mm	27 mm	25 mm	27 mm
***S. aureus* ATCC-25923**	20 mm	15 mm	12 mm	20 mm	14 mm	21 mm	20 mm	-

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

Amp=Ampicillin, Ami=Amikacin, Cipro=Ciprofloxacin, Cefo= Cefotaxim, Pipera = Piperacillin

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 *Staphyllococcus* 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 *Enteroccus* 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 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

***Enterococcus* (n=20)**	Diameter of zone of inhibition (mm)						
	AT	CA	HC	Nitro	Teico	Line	Vanco
Mean (SD)	21.15 (2.66)	12.65 (1.66)	15.45 (2.06)	21.85 (2.15)	19.35 (0.81)	22.35 (2.18)	12(1.25)
Median	20.5	12.5	15	22	19	23	12
Range	10	6	8	6	3	6	4

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

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 Teicoplanin

E. coli (n=20)	Diameter of zone of inhibition (mm)				
	AT	CA	HC	Nitro	Teico
Mean (SD)	12.30 (1.14)	11.85 (1.06)	20.45 (1.46)	22.05 (1.85)	11.10 (0.94)
Median	12	12	20	22	11
Range	4	4	5	5	3

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

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

S. aureus (n=20)	Diameter of zone of inhibition (mm)					
	AT	CA	HC	Nitro	Linez	Vanco
Mean (SD)	22.25 (2.27)	12.05 (1.24)	13.6 (1.35)	13.35 (1.38)	23.15 (1.52)	23.05 (1.77)
Median	22	12	14	13.5	23	23
Range	8	4	4	4	5	5

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 shown to be beneficial and important sources of new drug entities. These secondary plant metabolites proved to be effective and potential antimicrobial agents.[3]The phenolic compounds found in plants have been known to have antimicrobial characteristics.[13] The polyphenols along with sterols have been found to inhibit bacteria and fungi growth.[1] 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.[14] These secondary metabolites have been found to exhibit wide pharmacological activities like anticancer, antiviral, antimutagenic and anti-inflammatory activities.[15] 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 activit.[16] The presence of steroidal components has also been found to elucidate low antimicrobial activity against *S. aureus*, *E. coli*, *S. Typhimurium* and *C. Albican*.[17] 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.[18]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.[19] Antimicrobial activity of the ethanolic extract of *A.tagala* roots has been reported in strains of *Staphyllococcus* aureus.[20] 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 activities against microorganisms like *S. aureus* and *S. ureae* as well as a potential treatment option against *Salmonella*, *Brucella*, *Listeria*, *Bordetella* and *Helicobacter*.[21] 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*).[8] Traditionally, *C.asiatica* has been used as an antioxidant and antibacterial agent.[2] *C.asiatica* root and leaf extracts were reported to have antimicrobial activity in many bacterial isolates like *E. coli*, *Staphyllococcus* aureus, *Aspergillus flavus*, *Pseudomonas* and *Candida albicans*.[24] 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 *Staphyllococcus*. Roots of CA have also been studied and reported that it has more potential inhibitory action as compared to its leaves.[19, 23]

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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