

Anti-bacterial and anthelmintic effects of ethanolic leaf extract of *Aristolochia indica* L.

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Abstract

Objective: To explore the efficacy of ethanolic leaf extract of *Aristolochia indica* L. in bacterial and worm infections using experimental models. **Methods:** Preliminary phytochemical screening was evaluated by following standard procedure. Antibacterial activity was carried out by disc diffusion method and anthelmintic effect of the plant extract was determined based on time taken for paralysis and death of *Haemonchus contortus*. **Results:** Phytochemical analysis of the plant extract indicated the presence of carbohydrates, reducing sugars, combined reducing sugars, glycosides, tannins, alkaloids, acidic compounds, steroids, terpenoids and flavonoids. The extract showed activity against the bacterial strains namely *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus megaterium*, *Escherichia coli*, *Salmonella typhae*, and *Vibrio cholera* species at the dose of 250 µg/disc and 500µg/disc in comparison with standard drug Ciprofloxacin (5 µg/disc). In anthelmintic test, the extract showed significant dose dependent decrease in paralysis time and death time of *Haemonchus contortus*. **Conclusion:** These results suggest that the extract possesses significant anthelmintic and antibacterial effects that support ethnopharmacological uses of this plant.

Keywords: Anti-bacterial, anthelmintic, *Haemonchus contortus*, phytochemicals, *Aristolochia indica*.

1. Introduction

Aristolochia indica L., a member of the family Aristolochiaceae, is a creeper plant widely distributed in India, Sri Lanka and Bangladesh. It is commonly known as Ishwari, Nakuli and Gandhanakuli and has enormous therapeutic potential. This plant contains Aristolochic acid which is a rodent carcinogen and highly nephrotoxic and may be a causative agent in Balkan nephropathy. It has been found to be effective in the treatment of intermittent fever, malaria, parasitic infestations, various skin diseases, as an aphrodisiac, an anthelmintic and it is also used in oedema, intestinal disorders (Heinrich *et al.* 2009), fungal and bacterial infections (Shafi 2002, Kumar 2006). The plant has

been prescribed to treat cholera, bowel troubles, ulcers, leprosy, poisonous bites (Krishnaraju *et al.* 2005; Kanjilal *et al.* 2009). It is used as emmenagogue, abortifacient, antineoplastic, antiseptic, anti-inflammatory, antibacterial and phospholipase A2 inhibitor (Achari *et al.* 1981; Das *et al.* 2010). It is also traditionally used to treat different ailments including snake bites, inflammation, bronchial asthma, indigestion, blood pressure, gastric, scorpion stings and envenomation by other poisonous insects (Rastogi 2001).

Infections with helminths or parasitic worms affect more than two billion people worldwide. Helminthiasis or infections with parasitic worms are pathogenic for human beings. Immature forms of the parasites invade

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human beings via the skin or gastrointestinal tract (GIT) and evolve into well differentiated adult worms that have characteristic tissue distribution. Anthelmintics are the drugs that may act locally to expel worms from the GIT or systemically to eradicate adult helminths or development forms that invade organs and tissues. Most of the existing anthelmintics produce side effects such as abdominal pain, loss of appetite, nausea, vomiting, headache and diarrhea (Goodman *et al.* 2001). Chemotherapy is the only treatment and effective tool to cure and control helminth infection as effective vaccines against helminths have not been developed so far. Indiscriminate use of synthetic anthelmintics can lead to resistance of parasites (Singh *et al.* 2002). Herbal drugs have been in use since ancient times for the treatment of parasitic diseases in human (Chopra *et al.* 1956) and could be of value in preventing the development of resistance (Hammond *et al.* 1997).

The uses of this plant as traditional medicine confirm that it may possess some important biological activities. Previous scientific investigations have reported that different parts of this plant possess anti-diarrheal (Dharmalingam *et al.* 2014), antimicrobial (Kumar *et al.* 2011), anti-spermatogenic (Pakrashi and Pakrasi 1977), anti-fertility (Pakrashi and Pakrasi 1979), anti-oestrogenic and anti-implantation (Pakrashi and Chakrabarty 1978), anti-neoplastic (Rana *et al.* 2002), anti-inflammatory activity (Das *et al.* 2010), antioxidant (Thirugnanasampandan *et al.* 2008), anti-diabetic (Karan *et al.* 2012) activity but anthelmintic and anti-bacterial activities of ethanolic leaf extract of the plants have not yet been well explored. Therefore, the present study was undertaken to carry out the possible antibacterial and anthelmintic effects of the ethanolic leaf extract of *Aristolochia indica* L.

2. Materials and methods

2.1. Plant material collection and identification: The leaves of *A. indica* were collected from the Khulna district on 12th October, 2013 at the daytime. During collection, any type of adulteration was strictly prohibited. The plants were officially authenticated by the experts of Pharmacy Discipline, Khulna University, Bangladesh.

2.2. Preparation of crude extract: The collected leaves were separated from undesirable materials, shed dried and ground into a coarse powder with the help of a suitable grinder. 150 g of grinded leaves powder was soaked in 700 ml of ethanol in a glass container. After 15 days, the extract was separated from the plant debris by filtration using Whatman filter paper. The extract was concentrated by evaporation. The amount of yield in the extract was 14.67%.

2.3. Experimental animal: Live parasites *Haemonchus contortus* (Nematode) were collected from freshly slaughtered cattle at local abattoirs and identified by Dr Md. Royhan Gofur, Lecturer, Department of Animal Husbandry and Veterinary Science, Rajshahi University, Rajshahi, Bangladesh. After washing, parasites were stored in 0.9% phosphate-buffered saline (PBS), pH 7.4 (8.01 g NaCl, 0.20 g KCl, 1.78 g Na₂HPO₄ and 0.27 g KH₂PO₄ in 1 L dH₂O) at 37±1 °C.

2.4. Phytochemical screening test: Preliminary phytochemical analysis of ethanolic leaf extract of *A. indica* was carried out by employing standard procedure (Ghani 2003).

2.5. Evaluation of antibacterial activity by the disc diffusion method: Antibacterial activity of the ethanolic leaf extract of *A. indica* was measured by disc diffusion method followed by Biswas *et al.* (2014). Nutrient agar and Mueller-Hinton agar were sterilized in a flask and cooled to 45-50 °C and then taken in sterilized petri dishes with a diameter of 120 mm. The filter paper discs (5 mm in diameter) were impregnated with the crude extract at the concentration of 250 and 500 µg/disc and then placed onto the agar plates previously inoculated with the tested microorganisms. The tested microorganisms included *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus megaterium*, *Escherichia coli*, *Salmonella typhae*, and *Vibrio cholera*. The Petri dishes were kept at 4 °C for 2 hrs. The plates were incubated at 37 °C for 16 hrs to allow the growth of the microorganisms. The diameters of the zones of inhibition were measured in millimeters using a calibrated scale. All the tests were repeated in triplicate. Blank discs were used as negative control to ensure that the residual solvent's activity and the filter paper were not active by themselves. Ciprofloxacin was used as standard drug at the dose of 5 µg/disc.

2.6. Evaluation of anthelmintic activity: Anthelmintic activity of the leaves extract of *A. indica* was investigated on live parasites *H. contortus* of cattle as per the method of Akter *et al.* (2014) with minor modifications. The parasites were divided into different groups consisting of six parasites in each group. Plant extracts at the concentrations of 25, 50 and 100 mg/ml and albendazole at the concentrations of 10 and 15 mg/ml were prepared and transferred to petri dishes. Control group was treated with 0.1% tween-80 in PBS. Six parasites were placed in each Petri dish and observed. The time of paralysis was recorded when no movement was observed unless shaken vigorously. The death time was recorded after evaluating that the parasites did not move when shaken vigorously, dipped into warm water (50 °C) or subjected to external stimuli. Anthelmintic activity was expressed as the time

required for paralysis and death of parasites as compared to control..

2.7. Statistical analysis : The data were presented as mean \pm SEM (n=6). Results were analysed by One-way analysis of variance (ANOVA) followed by Turkey's multiple comparisons test. Student t test were used to compare between two groups. The significant difference was considered at $P < 0.05$.

3. Results

3.1. Phytochemical test: Preliminary phytochemical analysis of ethanolic leaf extract of *A. indica* showed the presence of reducing sugars, alkaloids, flavonoids, tannins and steroids..

3.2. Anti-bacterial test: Antibacterial effects of ethanolic leaf extract of *A. indica* against both gram positive and gram negative bacteria were qualitatively and quantitatively investigated by the presence or absence of inhibition zones. The plant extracts dose-dependently inhibited growth of bacteria (**Table 1**). The extract showed significant zone of inhibition of 21 mm and 30 mm with Gram negative *E. coli* at the dose of 250 μ g/disc and 500 μ g/disc respectively whereas the standard drug Ciprofloxacin showed zone of inhibition of 23 mm at the dose of 5 μ g/disc. In case of *Salmonella typhae*, the extract produced zone of inhibition of 20 mm (250 μ g/disc), 25 mm (500 μ g/disc) whereas the standard drug Ciprofloxacin showed zone of inhibition of 32 mm at the dose of 5 μ g/disc. Similarly with the Gram positive bacterial strain, zone of inhibition produced by the extract were 20 mm and 25 mm for *S. aureus* at the dose of 250 μ g/ disc and 500 μ g/disc respectively, and 8 mm and 17 mm for *S. epidermidis* at the dose of 250 μ g/disc and 500 μ g/ disc respectively whereas the standard drug kanamycin showed zone of inhibition of 27 mm and 30 mm respectively at the dose of 5 μ g/disc.

Table 1 Antibacterial effects of ethanolic leaf extract of *A. indica*.

Bacterial strains	Diameter of zone of inhibition in mm			
	Blank	Ciprofloxacin 5 μ g/disc	Extract 250 μ g/disc	Extract 500 μ g/disc
<i>S. aureus</i>	0	27	20	25
<i>S. epidermidis</i>	0	30	17	20
<i>B. megaterium</i>	0	30	0	15
<i>E. coli</i>	0	23	21	30
<i>V. cholerae</i>	0	35	0	20
<i>S. typhae</i>	0	32	20	25

3.3. Anthelmintic test: Anthelmintic effect of the plant extract was evaluated on the basis of paralysis and death time of *H. contortus*. As shown in **Table 2**, the plant extract demonstrated significant anthelmintic activity at the concentration of 25, 50 and 100 mg/ml in a dose dependent manner compared to standard drug Albendazole. The extract showed shortest time of paralysis and death of the parasites at 100 mg/ml. At the concentration of 100 mg/ml, the extract caused paralysis of the parasites at 691.5 sec and death at 354.8 sec while Albendazole caused paralysis and death at 527.5 and 630 sec, respectively at 10 mg/ml and 444.2 and 538.3 sec at 15 mg/ml, respectively. It is cleared that the time for paralysis and death decreases as the concentrations of the extract increases.

Table 2: Anthelmintic effects of ethanolic leaf extract of *A. indica*

Treatments Concentration (mg/ml)	Taken time for paralysis (seconds)	Taken time for death (seconds)
Control (0.2% Tween-80)	-	-
Albendazole, 10	527.5 \pm 10.98	630 \pm 13.49
Albendazole, 15	444.2 \pm 06.81	538.3 \pm 8.05
Extract, 25	1202.2 \pm 10.34****	1690 \pm 28.65****
Extract, 50	858.0 \pm 29.11****	438 \pm 9.70****
Extract, 100	691.5 \pm 18.57****	354.83 \pm 7.42****

Data are represented as mean \pm SEM, n=6. ****p<0.001, significant compared to Albendazole.

4. Discussion

In the present study, antibacterial effects of the plant extracts were evaluated using disc diffusion method. The tested bacterial strains were selected based on their clinical importance as they develop resistance against different antibiotics with their frequent uses. In consistence with Kumar *et al.* (2011), the plant extract showed effectiveness against both gram positive and gram negative bacteria which suggest that the plant extract may possess broad spectrum of antibiotic compounds or simply general metabolic toxin (Mohammed *et al.* 2010). The concentrations of the extract for bacteristasis were much higher than the concentrations of commercially available antibiotic Ciprofloxacin. This is explained by the fact that the crude form of plant extract possesses a lower concentration of bioactive compounds (Baravalia *et al.* 2009) and further purifications may yield more potent compounds.

It has been reported that phytochemicals like terpenoids, flavonoids, tannins, alkaloids, steroids and some phenolic compounds are responsible for the antibacterial activity of plant extract (Ramzi *et al.* 2008; Sule *et al.* 2011). Our preliminary phytochemical study revealed that the plant extract contains alkaloids, flavonoids, tannins and steroids. These phytoconstituents of the plant extract may be responsible for the antibacterial activity.

The plant extract also showed potent anthelmintic effects compared to standard drug Albendazole. This may be described by the fact that several compounds like alkaloid, polyphenol, flavonoid and terpene may be responsible for the anthelmintic activity of the plant (Bate-Smith 1962). The plant extract contains abundant of various phytochemicals among which some may be responsible for the wormicidal activity. These compounds may act on the CNS of the parasites causing paralysis and death of worms or interfere with the energy generation in the helminthes by uncoupling the oxidative phosphorylation or they bind to free proteins in the gastrointestinal tract of the host animal or to glycoprotein on the cuticle of the parasite and causes death (Salhan *et al.* 2010).

5. Conclusion

Ethanollic leaf extract of *A. indica* has potent antibacterial and anthelmintic effect that supports the traditional uses of this plant for therapeutical purposes. Further study is needed to be explored for its phytochemical profile to recognize the bioactive constituents accountable for its versatile activities.

6. Acknowledgement

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7. Conflict of interest statement

We declare that we have no conflict of interest.

8. References

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