

# In-Vitro Trial of Roots of *Ishwarmuli* (*Aristolochia indica* Linn.) for Anti-Microbial, Anti-Fungal & Anti-Oxidant Activity

## Research Article

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## Abstract

*Aristolochia indica* Linn. is a plant belonging to the family *Aristolochiaceae*. The medicinal value of *A. indica* has been known in different system of traditional medicine including *Ayurveda*. A number of *Aristolochia* species has been used in herbal medicines throughout the world for the cure of several ailments including metabolic diseases to venomous bites of snake and insects. The current work was executed with an objective to explore the in vitro antimicrobial, anti-fungal and anti-oxidant activity of *A. indica*. The relevant literature was also pursued for the justification and comparing the resemblances in the results. Aqueous and ethanolic extracts of roots of *Aristolochia indica* Linn. were tested for their inhibitory effect against 6 bacterial strains [*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae* & *Shigella flexneri*] and 3 fungi strains [*Aspergillus niger*, *Aspergillus fumigatus*, *Candida albicans*]. Agar-well method was used for the assessment of in-vitro antibacterial and antifungal activity of *A. indica* against selected standard bacterial strains. The minimum inhibitory concentration for each extract of various micro-organisms was also measured. Ethanolic extract of *A. indica* shows Minimum concentration (MIC) value of 50-100 µg/ml against selected bacterial strains which is better when compared to the aqueous extracts. Ethanolic extracts also showed moderate effects against the selected fungal strains, whereas the aqueous extracts failed to exhibit any effect even at higher concentrations.

**Key Words:** *Aristolochia indica* Linn, Anti-bacterial, Anti-fungal, Anti-oxidant, Ethanolic & Aqueous extract.

## Introduction

Nature has bequeathed humans and animals with numerous plants containing a variety of alkaloids and active principles that can cure ailments & promote health. These medicinal plants are also rich sources to develop potential secondary metabolites. Herbal extracts have been utilized worldwide for treating several ailments. Numerous studies have been carried out on various natural products screening their antimicrobial activity. (1-5)

The *Aristolochiaceae* is a family of flowering plants having seven genera and more than 450 known species belonging to the Order *Piperales*. One of the genera is *Aristolochia indica* Linn. are perennial shrubs, or herbaceous plants. Its common names are *Ishwarmul*,

*Snakeroot*, *Indian Birthwort*, *Iswarballi*. The leaves are simple, membranous and are arranged alternately along the stem on leaf stalks. The flowers are large to medium-sized, growing in the leaf axils. (6) They are usually bilaterally or radially symmetrical. *Aristolochiaceae* family plants are being used under traditional *Ayurveda* and *Unani* systems of medicine in many parts of the world. It is used to induce vomiting and to treat various poisons such as snake bites, scorpion stings and envenomation by other poisonous insects as well as intestinal parasite, swelling, menstrual irregularities, dropsy, low appetite, ulcers and fever. (7,8,9) The Roots, leaves and whole plant has been used in skin diseases. It is also used as an appetiser, aphrodisiac and anthelmintic medicine. The leaves and bark are used in diarrhoea and in intermittent fevers in children. Its roots comprise of aristolochic acid A, isoaristolochic acid, and allantoin. Aqueous extract of root shows strong gelatinolytic, collagenase, peroxidase and nuclease inhibitory activities, and prolongs survival of animals administered with Russell's viper venom. Ethanol and petroleum ether root extracts showed significant anti-inflammatory, mast cells stabilizing, and antipruritic activities. (10) As a part of traditional medicine, it is rubbed with honey and given to treat

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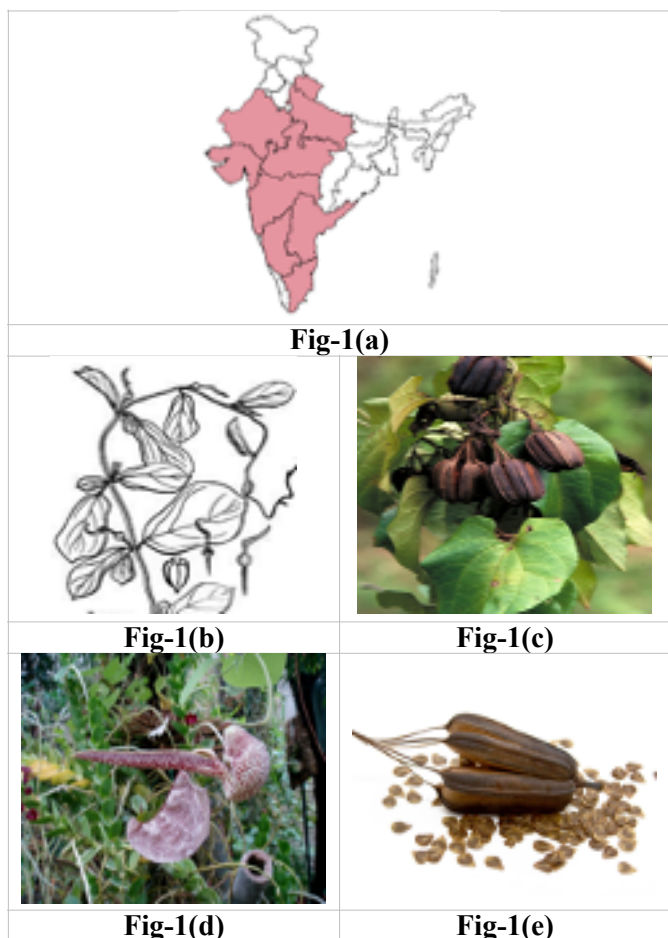
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Kustha (leprosy & other skin diseases). Keeping this in mind, present study was planned to evaluate in-vitro anti-microbial, anti-fungal and anti-oxidant activities of *A. indica*.

**Table 1: Botanical Classification of *Aristolochia indica* Linn.**

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Super division	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Magnoliidae
Order	:	Aristolochiales
Family	:	Aristolochiaceae
Genus	:	<i>Aristolochia</i> L.
Species	:	<i>A. indica</i>



**Figure 1: Showing Distribution & morphological characteristics of *Aristolochia indica* [1(a) Distribution, 1(b) leaves, 1(c) fruit, 1(d) flower, 1(e) seeds]**

**Distribution & Morphological Characteristics**

The species *Aristolochia indica* is distributed throughout the tropical, subtropical and Mediterranean countries. In India, plant is found in low hills and planes of south India, west Bengal and some part of north east. It is also available in Nepal, Bangladesh and coromandel coast. (11) It is extensively scattered in India, Nepal, Sri Lanka and Bangladesh. It is found all over the plains and low hilly part of India west Bengal, Bihar, Orrisa, Puri, Konkan and majority of districts of

south India. It is reported to be endangered species in northern part of India including Gujarat and Rajasthan. (12,13) *A. indica* It is usually found scrambling over hedges and bushes. [Fig. 1(a)].

Leaves of *A. indica* are alternate, entire with more or less undulate margins, somewhat cordate, acuminate or obovate. [Fig. 1(b)] Flowers comprise of light purplish perianth with inflorescence in axillary cymes or fascicles, 1-2 lipped, hairy within limbs dilated & having foetid odour [Fig. 1(d)] Fruit is globose, six valved capsule, quadrilateral, septicidal, and opening from below upwards. [Fig. 1(c)] Seeds are numerous, flat and winged. [Fig. 1(e)] (14).

**Materials and Methods**

**Collection of roots of *Aristolochia indica***

The roots of *A. indica* plant were procured from traditional herb vendor from the city of Kochin, Kerala, southwest India's coastal state. The plant material was authenticated at the Department of Dravyaguna, Government Ayurved College, Nanded, Maharashtra state, India. After authentication, the material was sterilized using 0.2% mercuric chloride and washed with distilled water to remove the surface dirt and soil particles. The material was then air-dried and then pulverized to obtain its coarse powder (40-80 mesh). The coarse powder was then stored in the desiccator to remove the moisture content.

**Extractions of *A. indica* [aqueous & ethanolic]**

Soxhlet extraction: Coarse powder of *A. indica* was placed in a porous bag made of strong filter paper in thimble chamber of the Soxhlet apparatus. Extraction solvent [i.e. distilled water & ethanol] was heated in the bottom flask, vaporized into the sample kept in thimble chamber, condensed in the condenser and drip back. When the liquid content was reached the siphon arm, the liquid contents emptied into the bottom flask again and the process was continued. (15) The the obtained extracts were concentrated by Flash rotary vacuum evaporator and the crude extracts were allowed to dry completely in petridishes. The dried crude extracts were preserved in sterile, air tight containers.

**Observations and Results**

The crude root powder was subjected to analysis for its physicochemical properties, antioxidant activity & total phenol contents.

**Table 2: Ash value. Antioxidant activity & total Phenols in crude roots of *A. indica***

Sr. No	Parameter	Value	Unit
1	Total Ash	14.8%	w/w
2	Acid insoluble ash	5.25%	w/w
3	Water soluble ash	1.98%	w/w
4	Total Phenols	34.5	mg / 100gm
5	Anti-Oxidant Activity in terms of Ascorbic acid	33.2	mg/100 gm
7	Total Plate Count	11x 10 <sup>4</sup>	cfu/g
8	Total Fungal Count	04 x 10 <sup>2</sup>	cfu/g

### Anti-microbial Activity

Antimicrobial activity of *A. indica* was determined by Agar well method. Minimum inhibitory concentration (MIC) was determined by Muller Hinton and Saboured Dextrose Broth were used as medium for bacterial and fungal strains correspondingly. (16) Positive control for bacterial culture was also carried out under the comparable condition by using Cefpodoxime dispersible tablets (10 mg / ml) and Fluconazole tablet (10 mg / ml) as control for fungal culture. The petri-dishes with the bacterial and fungal cultures were incubated at  $37 \pm 20^{\circ}\text{C}$  for 24 hour and 27

$\pm 20^{\circ}\text{C}$  for 48 hour respectively. The assessment of anti microbial activity was based on the measurement of diameter of inhibition zone formed in the petri-dishes. The experiment was repeated thrice and the results were taken as mean of three readings.

The results of antimicrobial activity of aqueous and ethanolic extracts of *A. indica* against selected human pathogens and their potency were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and zone diameters as given in the Table 3.

**Table 3: Zone of Inhibition of Aqueous and Ethanolic extracts of *Aristolochia indica* Linn**

Sr. No	Organism	Zone of inhibition for Standard (Cefpodoxime)	Extract of roots of <i>A. indica</i> Concentration & Zone of inhibition (mm)					
			Extract	60%	70%	80%	90%	100%
<b>Anti-Bacterial Activity</b>								
1	<i>Staphylococcus aureus</i> (MTCC 96)	27.6 ± 0.34	A E	20.4 ± 0.2 21.5 ± 0.3	21.0 ± 0.4 22.3 ± 0.5	21.8 ± 0.3 23.9 ± 0.7	23.4 ± 0.5 24.8 ± 0.6	25.0 ± 0.3 26.4 ± 0.31
2	<i>Bacillus subtilis</i> (MTCC 441)	12.03 ± 0.5	A E	-- --	18.6 ± 0.4 19.1 ± 0.2	18.9 ± 0.6 19.9 ± 0.5	19.6 ± 0.76 20.2 ± 0.6	20.8 ± 0.54 20.9 ± 0.35
3	<i>Escherichia coli</i> (MTCC 443)	26.9 ± 0.6	A E	-- 18.4 ± 0.3	-- 19.6 ± 0.5	17.6 ± 0.5 22.8 ± 0.7	18.7 ± 0.7 24.9 ± 0.3	19.5 ± 1.37 27.9 ± 1.45
4	<i>Salmonella typhi</i> (MTCC 733)	39.5 ± 0.2	A E	-- --	-- --	-- --	12.1 ± 0.5 --	12.7 ± 0.4 --
5	<i>Klebsiella pneumoniae</i> MTCC 39	19.3 ± 0.3	A E	-- 18.5 ± 0.3	-- 18.9 ± 0.5	-- 19.7 ± 0.6	10.7 ± 0.2 21.8 ± 0.5	11.9 ± 0.5 22.1 ± 0.5
6	<i>Shigella flexneri</i> (MTCC 1457)	36.6 ± 0.4	A E	18.5 ± 0.5 19.0 ± 0.4	19.7 ± 0.4 19.9 ± 0.7	20.3 ± 0.5 20.9 ± 0.3	24.6 ± 0.6 22.6 ± 0.5	24.7 ± 0.4 23.5 ± 0.4
<b>Anti-fungal Activity</b>		<b>Fluconazole</b>		<b>60%</b>	<b>70%</b>	<b>80%</b>	<b>90%</b>	<b>100%</b>
6	<i>Aspergillus niger</i> (MTCC 1344)	20.5 ± 0.5	A E	-- --	-- --	-- 10.5 ± 0.4	-- 12.3 ± 0.3	-- 14.5 ± 0.6
7	<i>Aspergillus fumigatus</i> (MTCC 1344)	24.5 ± 0.4	A E	-- --	-- --	-- 13.1 ± 0.3	-- 14.4 ± 0.5	-- 16.5 ± 0.5
8	<i>Candida albicans</i> (MTCC 227)	41.3 ± 0.3	A E	-- --	-- --	-- 18.7 ± 0.5	-- 18.9 ± 0.5	-- 20.5 ± 0.6

A: Aqueous extract of *Ishwarmuli Churna* (*A. indica*)

E: Ethanolic extract of *Ishwarmuli Churna* (*A. indica*)

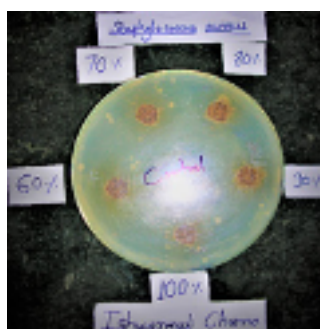


Fig-2 (a) *Staphylococcus aureus*

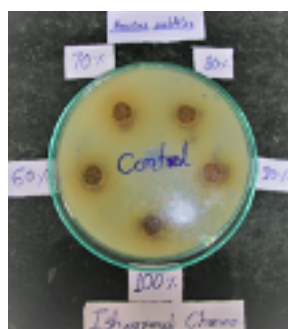


Fig-2 (b) *Bacillus subtilis*

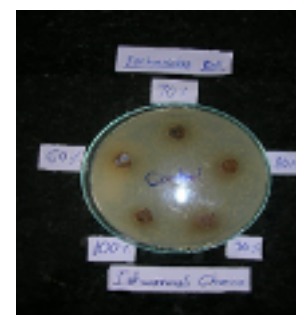


Fig-2 (c) *Escherichia coli*



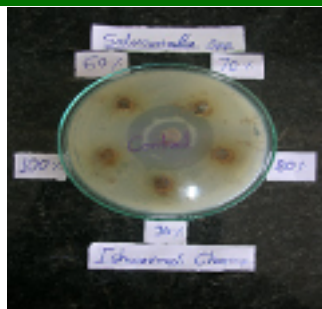


Fig-2 (d) *Salmonella typhi*



Fig-2 (e) *Shigella flexneri*

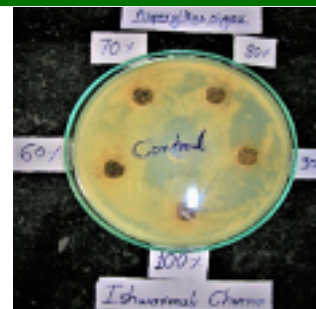


Fig-2 (f) *Aspergillus niger*



Fig-2 (g) *Candida albicans*

Figure 2: Showing Results of ethanolic extracts of *Aristolochia indica* [2 (a) *Staphylococcus aureus*, 2 (b) *Bacillus subtilis*, 2 (c) *Escherichia coli*, 2 (d) *Salmonella typhi*, 2 (e) *Shigella flexneri*, 2 (f) *Aspergillus niger*, 2 (g) *Candida albicans*]

## Discussion

Infectious diseases are one of the main cause of morbidity and mortality throughout the world. The number of multi-drug resistant microbial strains are growing successively and there is reduced susceptibility to antibiotics is noted. This is a prospective situation for pursuing the new antimicrobial substances from available herbal sources. (17) Plant derivatives such as extracts are being used for preparing various dosage forms. Aqua or water is primary solvent used for preparation of various herbal dosage forms. In many studies it was found that the plant extracts extracted in organic solvents also have profoundly distinct antimicrobial activity compared with aqueous extracts. (18) Hence, in the present work, extract was prepared using water & ethanol and compared. Initially estimation of the total residual ash and moisture content of the shade-dried powdered were carried out using the standard procedure mentioned in Ayurvedic Pharmacopeia of India. The values of the physicochemical tests were as per the parameters mentioned in API which indicated plant material was pure and devoid of any adulterants. The total antioxidant capacity of ethanolic extracts was evaluated by the method of Prieto et. al. by using ammonium molybdate reagent and a spectrophotometer and was compared to that of ascorbic acid. Quantitative analysis of antioxidant activity of *A. indica* was attributed as the number of gram equivalents of ascorbic acid. (19) Total polyphenolic contents were assessed by using the method of Folin–Ciocalteu reaction, with tannic acid. (20, 21) The presence of phenols indicates that roots of *A. indica* as well as its ethanolic extract is antioxidant in nature. Further it was observed that the ethanolic extract of *A. indica* significantly inhibit the growth of

microorganisms such as bacterias and fungi. Ethanolic extracts are showing marginally better results as compared to the acqueous extracts in all selected bacterial strains except *Salmonella typhi* (MTCC 733); where acqueous extracts are effective at higher concentrations. Further ethanolic extracts showed moderate effects agains the selected fungal strains, whereas the acqueous extracts failed to exhibit any effect even at higher concentrations. Ethanol extract of *A. indica* shows better Minimum inhibitory concentration (MIC) value of 50-100 µg/ml against selected bacterial strains as compared to the acqueous extracts. Antimicrobial activity of *A. indica* might be due to the phytochemical components present in it. There are documentary evidences that shows ethanol is a better solvent for consistent extraction of antimicrobial substances from medicinal plants over water which contributes in added antimicrobial activity of the ethanolic extract over water extract. (22, 23).

### Limitations of the Study:

- The method of Soxhlet extracts is considerably primitive and further purifications by more sophisticated methods may yield more potent compounds.
- The study was conducted against the single control drugs i.e. Cefpodoxime for bacterial strains and Fluconazole for fungal strains. Further studies should also be considered against alternative and more suitable anti-bacterial and anti-fungal drugs as control.

### Conclusion

*A. indica* is one of the most important medicinal plants used Ayurveda medicines because of

its numerous pharmacological properties. Ethanolic extract of *A. indica* showed significant anti-microbial activity against selected gram positive and gram negative bacteria. The detection of antimicrobial activities in present research, although at varying degrees indicates that *Aristolochia indica* may be a potent sources for bactericidal and fungicidal drugs. Present research will help in further development of the drug and thereby its application in interrelated conditions.

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