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Anthelmintic activity of aristolochia indica linn. Leaves

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ABSTRACT:
Aristolochiaindica L. (Family: Aristolochiaceae) has long been used in Indian subcontinent in the traditional system of medicine to treat cholera, fever, bowel troubles, ulcers, leprosy, skin diseases, menstrual problems and snakebites. *Aristolochiaindica (Linn)* commonly called Iswarmul is a rare endangered medicinal plant of India. The present research programme was aimed to investigate the anthelmintic activity of the aqueous , ether and ethanol extract of *Aristolochia indica.*The anthelmintic activity was evaluated on adult Indian earthworm Aristolochia indica linn. due to its anatomical and physiological resemblance with the intestinal parasites of human being. Five earth worms of nearly equal size were placed in known standard and test extract solution at room temperature. The compound were evaluated by the time taken for complete paralysis and lethal time for test compound was recorded and compared with that of known standard. *Aristolochiaindicalinn.*leaf is sensitive to the majority of anthelmintic drugs that are used against parasitic worm infections of humans and livestock. Effect of Aqueous , Ether and Ethanol extracts of*Aristolochia indica linn.* leaf has anthelmintic activity in dose dependent. higher concentration of ether extract showed maximum effect when compared with ethanol, aqueous extract.The Ether, Ethanol and Aqueous extract of aristolochia indica linn. leaf have been confirmed to display anthelmintic activity.

INTRODUCTION:[1,2,3,4]
Aristolochiaindica Linn. A member of aristolochiaceae, is one of the most wide used in india.it is also rare and endangered medicinal plant. It is shrubby twining: stems long; slender woody as the base, grooved, glabrous, leaves variable; flowers in few-flowered axillary racems: bracts small ovate acuminates, opposite the pedicels; seed deltoid ovate, acute, flat, winged." It is heart shape" .it is roots are widely used in pungent, pains in the joint, the seed are useful in inflammations, laves juice are useful anthelmintic activate. From the time immemorial, plants have been widely used as curative agents for variety of ailments. World Health Organization has listed 21,000 plant species used around the world for medicinal purpose. In India about 2,500 plants species belongs to

more than 1000 genera are being used in the indigenous system of medicine. India is tenth among the plants rich countries of world and fourth among the Asian countries. Aristolochiais a large genus comprising more than 800 Species, most of them twining lians, and is widely distributed in tropical and subtropical regions almost all over the world Aristolochic acid is mainly found in the genus Aristolochiathe compound contains organic nitro groups. They are derived from benzylisoquinoline alkaloid precursors. Well documented in Ayurveda and Unani system of medicine to treat different ailments.

Aristolochiaindicalinn in traditional medicine, is used as ahemostatic, usually to tea to treat microbial infections. However, in Benin, no scientific study has proved hemostatic potential of this plant. It is then what justifies the study entitled "hemostatic activity screening and skin toxicity of sap of aristolochiaindicalinn. Used in traditional medicine (Benin)"

The Aristolochiales are a group of paleoherbs, a basal group flowering plants. The plant is commonly known by the names birthwort & snakeroot, which refers to its use in traditional medicine for postpartum infections and snakebite respectively. AristolochiaIndicais the plant belonging to family Aristolochiaceae, different parts of the plant bears many medicinal properties. The plant has been used in skin diseases where there is morbidity of vata, pitta &kapha. It is used as an appetizer, aphrodisiac &anthelmentic. The fresh juice of the leaves is a popular antidote to snake poison. The leaves & barks are used in bowel complaints of children, diarrhea& in intermittent fevers. As a blood purifier the dried roots & rhizomes are used as a gastric stimulant & bitter tonic. The root is used in skin diseases. It heals wounds & destroys the toxic effect of all poisons. In olden days, it was used against snakebites. In traditional medicine the underground parts of the plant are rubbed with honey & given to treat leprosy & macerated with black pepper. The roots & stems of the plant are used in ethno veterinary aches and pains, rheumatism, anthrax, madness, antibacterial effect**5**, antineoplastic effect, ant arthritic& snakebite. The major chemical constituents present in the aristolochia extracts are PhenanthrenederivativeslikeAristolochicacidaristolochic acid–D, aristolochic acid–D methyl ether lactum, aristo lactum β-D glucoside/aristolic acid, aristolic acid, methyl ester, methyl aristolochate, aristo amide, aristolinic acid, aristolonitrite. Quinones like Aristolindiquinone, lactones like Aristololide, Alkaloid like Aristolochine, Terpenes like mono &sesquiterpenes including linalool, β –caryophyllene, α-humulene, ishwarone, caryophyllene oxide, ishwarol, ishwarane&aristolochene& α-terpinolene.

The extracts are found have various medicinal activities like, Antiestrogenic activity, Abortifacient activity, Interceptive activity. Antitumor activity, ant fertility, Immunomodulatory activity and anti-inflammatory activity. Among that the leaves were predicted to have antihyperuricemic effect according to folklore claim. Hyperuricemia is increased uric acid concentration in blood having drug induction as one of the reasons. Aristolochiaindica leaf extracts were said to have influence on this drug induced gout. Drug induced gout or hyperuricemia is a common problem nowadays. Reports suggest that diuretic drugs like amiloride, ethacrinic acid, frusemide and thiazides in ducehyperuricemion high doses and on prolonged use. Hence there is a search for antihyperuricemic drugs that can effectively control the diuretics induced Hyperuricemia. It was also found that various drugs act by controlling the hyperuricemia, such as Allopurinol that was found to act by inhibiting the synthesis of uricacid**15** and probenecid like uricosuric that act by increasing the rate of excretion of uric acid level, NSAIDS are found to reduce the pain and inflammation associated with gout. In this work qualitative activity of anti hyperuricemic effect of Aristolochiaindicawhole plant extracts by comparing with standard uricosuric agent like probenecid.

AristolochiaindicaLinn:[4,5]
a native of India and is commonly named as Iswarmul . It is a rare endangered medicinal plant. It is a twining herb, semiwoody, leaves are cordate or ovate, estipulate; flowers are irregular, often offensively smelling, perianth is globose with a purple dilated and trumpet-shaped mouth with a strap-shaped brown purple appendage or lip behind; fruit is a subglobose capsule. Its roots are widely used in inflammations, biliousness, and dry cough. It is good for snake bite also. The leaf of the plant or the roots of the plant is said to be a specific antidote for cobra poisoning. It is also reported to be a stimulant, and used for diarrhea and intermittent fever. This plant is also a remedy for dropsy and loss of appetite. The present investigation attempts to evaluate the antiglycemic effect of *A.indica*. And also to evaluate its toxicity Chalconeswereon liver function, kidney functions

Morphology:
Leaves: Variable, in the broad form 10-12.5 by 7.5 cm; in the narrow form 3.8-10 by 1.3 to 2.5 cm from linear

oblong to obovate –oblong or sub-panduri form, usually obtusely acuminate, glabrous, entire with somewhat undulate margines, base cuneata, rounded, subtruncate or subcordate.
Flowers: Flowers are few-flowered axillary racemes; bracts small, ovate, acuminate, opposite the pedicels; pedicles long, thickened above perianth greenish white, reaching 4.5 cm long, with globose inflated base, then bent at a right angle and suddenly narrowed into a cylindric tube.
Seeds: Deltoid-ovate, acute, flat, winged.

Fig 1: Aristolochiaindica Linn Leaves

Phytochemistry:[7]
Acidic and basic constituents of *A. indica* were reported while discussing the chemistry of the *Aristolochia* species. Ishwarol, a new tetracyclic sesquiterpene alcohol from the plant species was reported by. have reported total synthesis of Racemic Ishwarane a tetracyclic sesquiterpenoid. 5βH,7β, 10α-Selina-4 (14), II-diene, a new sesquiterpene hydrocarbon from *A. indica* was discovered . Have reported new phenanthrene derivatives from the plant which included aristolochine alkaline, isoaristolochic acid, allatonin etc. A short synthesis of ishwarone was reported by. Synthesis of tetra cyclic sesquiterpenoids racemic is hwarone was reported by. (12S)-7, 12-Secoishwaran-12-ol, a new type of sesquiterpene was reported by. Aristololactam N-β- D-glucoside (a phenanthrene derivative) and 3β-hydroxy-stigmast-5-en-7-one and 6β-hydroxy-stigmast-4-en-3-one (two steroids) have been isolated from *A. indica* by. The roots of *A. indica* contains aristolindiquinone, aristololide, 2-hydroxy-1-methoxy-4Hdibenzo quinoline-4,5-(6H)-dione, Cephradione, aristolactamIIa, β-sitosterol-β-D-glucosidearistolactam glycoside I, stigmastenones II and III, methylaristolate, ishwarol, ishwarone and aristolochene The Aristolochic acids and Aristolactams were reported by. Aristolindiquinone, a new naphthoquinone from *A. indica* was reported.

Varieties:[8,9]
Aristolochiaindica linn.nana is a dwarf variety of *Aristolochia indica* popularly planted as an ornamental plant in gardens and larger containers, and used as a bonsai specimen tree. It could well be a wild form with a distinct origin. It differs in having green leaf, fruit blackish flower.

Uses of Aristolochia indica linn:
Used in Indian subcontinent in the traditional system of medicine to treat cholera, fever, bowel troubles, ulcers, leprosy, skin diseases, menstrual problems and snakebites. The plant is also used as emmenagogue, abortifacient, antineoplastic, antiseptic, anti-inflammatory, antimicrobial, antipyretic, antifertility and antispermatogenic agent. Aristolochic acid, a major active constituent of the plant is reported to cause cancer, nephropathy, sister chromatid exchange and is a potent abortifacient. The present review deals with the different scientific studies and reports available in different aspects of this plant in the areas of Morpho-taxonomy, Phytochemistry, Pharmacology, Medicoethnobotany, Tissue culture and Chromosomal study.

Ayurvedic identity:[10]
To trace out the identity of these twin plants with Ayurveda is very interesting and discussive. The twin plants described as 'Nakulidwaya' in Ayurveda, were brought under a comparative study with 'Arayadwaya' of kani tribes. The word 'dwaya' shows the twin species of plants belonging to the same family or different families with almost similar properties or possessing less or high therapeutic value.
ANTHELMINTIC:
Introduction:[11]
The term anthelmintic frequently is restricted to drugs acting locally to expel parasites from the GI tract. However, there are several types of worms that penetrate other tissues; drugs that act on these parasitic infections are also known as anthelmintics. Further more, drugs that kill worms are refer to commonly as vermicides; those that effects the worms in such a manner that peristaltic activity or catharsis expels it from the intestinal tract are referred to as vermifuges. This arbitary division serves no useful purpose because many anthelmintics manifest both

actions, according to the dose employed. Therefore, the anthelmintics are defined more properly as drugs used to combat any tye of helminthasis. The worm parasits of man belong to two phylaL: Nemathelminthes (round worms) and platy helminthes (flat worms), The round worms include tha hook worms, round worms , whip worm, pin worm,strogylodes steroralis, trichinella spiralis, and wuchereria bancrofti.

Drug usedas an antihelmintic:

1. Albendazol.
2. Mebendazle.
3. Piperazine.
4. Thiabendazole.
5. Praziuantel.
6. Diethylcarbamazine citrate
7. Pyrantel pamoate.

❖ **Albendazole**[12]

Preparation:

Etherification of 4-Marcaptoaretanilide with n-propyl bromide yields 2- nitro-4 (propylthic) acetanilide, which is hydrolyzed to the amine, reduced to the diamine with stanous chloride, then converted to the benzimidazoed struchure with S-methylthiourea, and finally acylated at the 2-amino group with methylcholoformate.

Description: Colourless crystals melting about 2090 (decompn).

Solubility:

In soluble in water; soluble in dimethylsufoxide (DMSO), acetic acid, strong acids, or bases; can be regenerated from these solutions by neutralization if not heated or kept for too long a time.

Anthelmintic activity:[13]

The anthelmintic activity was performed according to the method of Ghosh et al. on adult Indian earthworm Pheritimaposthuma as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human being .In the 50ml of formulation containing different concentration of Aqueous and methanol extract (50,100mg/ml in saline water) and standard (20mg/ml) were prepared and approximately equal size five earthworm were released in each group. Observations were made for the time taken to paralysis or death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body color. Albendazole (20mg/ml) was used as standard while normal saline as control. Treatment with an anthelminthic drug kills worms whose phenotype renders them susceptible to the drug. Worms that are resistant survive and pass on their "resistance" genes. Resistant worms accumulate and finally treatment failure occurs. See drug resistance.2 an anthelmintic drug. An anthelmintic may interfere with the parasites' carbohydrate metabolism, inhibit their respiratory enzymes, block their neuromuscular action, or render them susceptible to destruction by the host's macrophages. Drugs used in treating specific helmintic infections include piperazine, pyrantelpamoate, pyrviniumpamoate, mebendazole, niclosamide, hexylresorcinol, diethylcarbamazine, and thiabendazole.

Table 1. Key drugs registered for the treatment of parasitic worms in humans.

Schistosomiasis (blood fluke)	**Intestinal round worms**
Antimonies'	Piperazine
Metrifonate	Benzimidazoles
Oxamnaquine	Morantel
Praziquantel	Pyrantel
	Levamisole
Cestodiasis (tape worm)	Avermectins and milbemycins
Niclosamide	Closantel (and halogenated salicylamides)
Benzimidazoles	Emodepside
Praziquantel	
Fasciolasis (liver fluke)	**Filariasis (tissue round worms)**
Praziquantel	Diethylcarbmazine
Closantel	Suramin

Methodology:

This chapter deals with the methods utilized to perform different experiments to achieve said goals.

Procurement of plant material:
The plant *Aristolochiaindicalinn*. Was collected from local area of Duand dist.- Pune, State-Maharashtra.

Identification and authentication:
The herbarium of this plant was identified &authentify at Botanical Survey Of India (BSI) Office Pun by Dr. V.K.Rawat, (Scientist"C"& H.O.O) department of Botany.

Macroscopic study of plant material:
External features, dimensions and organoleptic properties of plants were studied.

Table 2. Microscopy of *Aristolochiaindica L.*

Parameter	Observation
Part	Leaves
Arrangement	Opposite
Size	7-10 cm long, 3-10 cm wide
Color	Light Green
Odour	Piperales
Taste	Bitter

Fig 2: Leaves of *AristolochiaIndica* L

Determination of physiochemical constants of powdered plant materials[14]
Ash values:
Determination of total ash, acid insoluble ash, and water soluble ash was carried out using reported methods in book.

Extractive Values:
Determinationof alcohol soluble extractive value and water soluble extractive value were carried out using reported method.

Preparation of extract:
Fresh *Aristolochiaindicalinn.*Leaf will be collect and air dry in shade at room temperature. The powder leaf material will be extract with water, ether and ethanol by maceration for 72 hrs. The extract will be dry at low temperature under reduce pressure.

- The plant materials were dried and powdered. The powder obtained was weighed then used for extraction.
- Ether, Ethanol and Water extracts of Aristolochiaindica linn. (Leaves) were prepared by soxhlet extraction.
- The resulting extracts were concentrated under reduced pressure using rotary vacuum evaporator and were transferred in petridishes and allowed to dry in vacuumed oven.
- The amount of extract were weighed and stored in airtight bottles.

Chemical: Ether , Ethanoland Aq. Extrict , Dist. Water , Std.Extict.
Appratas: Pitra Dish , Stop Wash.
Anthelmintic activity:[15]
The anthelmintic activity was performed according to the method of Ghosh et al. on adult Indian earthworm *Pheritimaposthuma* as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. *Pheritimaposthuma* worms are easily available and used as a suitable model for screening anthelmintic drugs. In the 50ml of formulation containing different concentration of Aqueous, Ether and Ethanol extract (50,100mg/ml in saline water) and standard (20mg/ml) were prepared and approximately equal size five earthworms were released in each group. Observations were made for the time taken to paralysis or death of

individual worms. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body color. Albendazole (20mg/ml) was used as standard while normal saline as control.

RESULT:

The *Aristolochiaindicalinn.* Leaf are performing Anthelmintic activity is earth worm successful.

Table 3. Microscopy of *Aristolochiaindica L.*

Evaluationproperties	Observation
Color	Light Green
Odour	Piperales
Taste	Bitter
Nature	Crystalline
Total ash vale	40%
Acid insoluble ash vale	25.4%
Water soluble ash vale	2.5%
Arrangement	Opposite

PHARMACOGNOSTICAL STUDIES:

Effect of Ether, Ethanol and Aqueous extracts of leaf of *Aristolochia indica linn* on worms

Experimental data showed that, the Aqueous, Ether and Ethanol extracts of *Aristolochia indica linn*. Has anthelmintic activity in dose dependent manner as shown in table no 3. The shortest time required for paralysis and death was observed with concentration of 50mg/ml. higher concentration of ether extract showed maximum effect when compared with 100mg/ml of ethanol extract.

Preparation of extract of Leaf of *Aristolochia indica Linn.* by maceration has yielded

Table No.4

Sr. No.	Solvent	Color & Consistency	Percentage Yield
1.	Ether	Brown and waxy	2.5%
2.	Ethanol	Brown and waxy	4.75%
3.	Water	Brown and Waxy	6.5%

Table No. 5

Anthelmintic activity of Aqueous, Ether, and Ethanol leaf extract of Aristolochia indica *linn.*

Test substance	Concentration(mg/ml)	Time taken for paralysis(min.)	Time taken for Death(min.)
Vehicle	-	-	-
Albendazole	20	15.44±0.14	19.48±0.13
Aqueous extract	50	42.54±0.17	50.43±0.112
	100	48.11±0.008	56.11±0.13
Ethanol extract	50	38.30±0.13	44.24±0.06
	100	21.44±0.05	29.34±0.07
Ether extract	50	20.22±0.04**	24.38±0.06**
	100	18.34±0.05**	22.10±0.09**

Values are expressed as MEAN±SEM, One way ANOVA followed by Dunnett's test n=5 in each group **P<0.01

ANTHELMINTIC ACTION:

Fig 3: Standard Albendazole

Fig 4: Control Dist Water

Fig 5: Ether (50mg/ml)

Fig 6: Ether (100mg/ml)

Fig 7: Ethanol(50mg/ml)

Fig 8: Ethanol(50mg/ml)

Fig 9: Aqueous (50mg/ml)

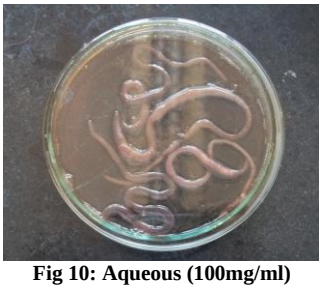

Fig 10: Aqueous (100mg/ml)

DISCUSSION:

Adaptation of locally available herb or herbal product in the treatment of human aliment of great advantages as far as cost availability of treatment concern. Hence the present study planned to investigates utility of locally and abundantly available plant that is *Aristolochiaindicalinn*. *In*treatmenthelminthes infection.

Albendazol by increasing chloride ion conductance of worms muscle membrane produced hyperpolarization and reduced excitability that which led to muscle relaxation and flaccid paralysisTheEther and Ethanol extracts of *Aristolochiaindicalinn*.Not only showed paralysis but also caused death of worms at different concentration.

The result indicates when concentration increases anthelmintic activity increases, and shortest time required for paralysis and death was observed with Ether extract when compared with Ethanol extract of*Aristolochiaindicalinn.*

Our study is not conclusive about the attribution of the activity to a particular phytochemical constituent, therefore further studies are needed to isolate, characterize and screen so as to establish lead molecule.

CONCLUSION:

The present study has shown that, the Aqueous, Ether and Ethanol extract of *Aristolochiaindicalinn.* have been confirmed to display anthelmintic activity. Further studies are in progress to identify the possible chemical constituent responsible for anthelmintic potential.

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