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Pharmacognostical, phyotochemical and toxicity profile of flower of *Ishwari* - *Aristolochia indica* Linn.

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Abstract

Aristolochia indica Linn. (Aristolochiaceae) is popularly known as *Ishwari* and Nakuli in Samhitha Granthas. Ishwari is used in different forms for condition like sheethajwara, sarpavisha, vrana, ekangashopha, unmada, apasmara etc. One of methods of plant wealth conservation is by promoting use of plant parts other than root in therapeutics. Flower is one such plant part which can be studied as substitute for therapeutic benefits of root. Prior to such trials such proposed substitutes must undergo various pharmacognostical, analytical, toxicological and pharmacological studies. Microscopy, phtochemical examination, HPTLC and acute toxicity studies of *A. indica* flowers were performed flowing standard procedure. Powder microscopy revealed some specific characters for its identification. Phytochemical study showed presence of steroid, carbohydrate, flavonoids and saponins. HPTLC fingerprint of the flowers was derived. On toxicity evaluation no adverse effects were observed on administration of powder of flower orally in rat. These diagnostic features can be used as a fingerprint for the identification and differentiation of their substitute and adulterants of the plant. As there is no toxicity up to 2000 mg/kg body weight, 1/10 of the dose i.e. 200 mg/kg can be considered as therapeutic dose for further studies on this drug.

Keywords: Microscopy, Phytochemical, HPTLC, OECD, Substitution.

Introduction

The drug *Aristolochia indica* Linn. from Aristolochiaceae family is popularly known as *Ishwari*. The drug *Nakuli* which has been mentioned in *Samhitha Granthas*; the *source* of which could also be equated to *A. indica* Linn. The drug is prescribed for the preparation of *taila* (medicated oil) for *sheetha jwara* (fever with rigor) and *ghrta* (medicated ghee) for *jwara* (fever), *unmada* (schizophrenia) and *apasmara* (epilepsy) in *Caraka samhita*[1]. In *Sushruta samhita* it has been mentioned for the preparation of lepa for *Sarpa visha* (snake poison) [2]. *Acharya Vagbhata* used this drug as *lepa* for *ekanga shopha* (local edema) and preparation of oil for *sheetaja* jwara (fever with cold) [3]. *Guna Karma* (properties) of the drug is mentioned in *hareetakyaadi varga of Bhavaprakasha* where the drug is said to be effective in wound healing process[4]. The plant root is said to be useful in the management of intermittent fever, children's bowel complaint and most extensively the root and leaf is used in the treatment of snake bite poisoning[5].

There are different methods of plant wealth conservation. One of such methods is by promoting use of other plant parts other than root in therapeutics. Flower is one of them which can be studied for its therapeutic benefit as substitute for root. But prior to such trial it has to undergo detailed standardization, chemical analysis and pharmacological property evaluation.

Medicinal plant materials are being adulterated in commerce due to many reasons such as similar morphological features, same vernacular or classical name, presence of similar active principles etc. The practice of substitution and adulteration will badly affect the therapeutic activity of herbal products. Therefore systematic identification of drugs and their substitutes is an essential step while producing standardized herbal products[6]. In the present study standardization and toxicity evaluation of flowers of *A. indica* was performed. Preliminary phytochemical study and HPTLC of flower are documented. Acute toxicity study was conducted to find its effective dose for further pharmacological activity evaluation.

Materials and methods

Plant materials

The authentic sample of flower of *Aristolochia indica* Linn. was collected from Udupi district of Karnataka, India. It was identified and authenticated by comparison with the botanical description mentioned in Flora[7]. The flowers are shade dried and the voucher specimen (No. 255/13051008) was

deposited at the Pharmacognosy Laboratory of S.D.M Centre for Research in Ayurveda & Allied science Udupi for future reference. For powder microscopy the samples were dried in shade, powdered and sift through mesh 40; the powder was stored in glass vial until microscopic evaluation. Coarse powder was used for the phytochemical examination and HPTLC study. For acute toxicity studythe test drug was made in to fine suspension in gum acacia and given orally.

Method

Powder microscopy was performed with a pinch of fine powder mounted on a microscopic slide with a drop of glycerin-water. Characters were observed using using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of the figures are indicated by the pre-calibrated scale-bars using Zeiss Axio Vision software[8].

Preliminary phytochemical investigation was done with the coarse powder to detect the presence of alkaloids, carbohydrate, triterpenoid, steroid, tannins, glycosides, flavonoids and coumarin in alcohol extract[9, 10].

one gram of powdered samples were dissolved in 10 ml ethanol and kept for cold percolation for 24h and filtered. 3, 6 and 9µl of the above samples of were applied on a pre-coated silica gel F_{254} on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in toluene: ethyl acetate (8: 1). The developed plates were visualized in UV 254, 366 nm and then derivatised with vanillin sulphuric acid reagent and scanned under UV 254nm, 366 nm. R_f, colour of the spots and densitometric scan were recorded[11, 12].

Acute oral toxicity flower was performed following OECD-425 guidelines using AOT software. Albino rats of either sex selected by random sampling were used for acute toxicity study. The animal were kept fasting for overnight and provided only with water. The test drug was administered at a dose of 175, 550 up to 2000 mg/kg (up and down method) and observed for 14 days. If any mortality was observed the same dose repeated again to confirm its toxic potential. If mortality was not observed the procedure was repeated for higher doses[13].

Result and Discussion

Powder microscopy

Powder of flower showed characters like thick walled bent and pointed tipped bicellular trichome with content in the foot cell; polygonal thick-walled slightly beaded cells of the epidermis of stalk of the flower in surface view; round to oval parenchyma cells of the cortex having intercellular spaces; longitudinally cut fragment of stalk of flower with vascular strands and attached parenchyma; transversely cut fragments of perianth with thick walled epidermis having mesopyhll tissue underneath it; fragment of collenchyma tissue underlying the midrib region of the perianth; plenty of fragments of mesophyll cells with reddish brown content; sclerified anther fragments and round to oval pollen grains with rough exine are scattered throughout the powder (Figure 1).

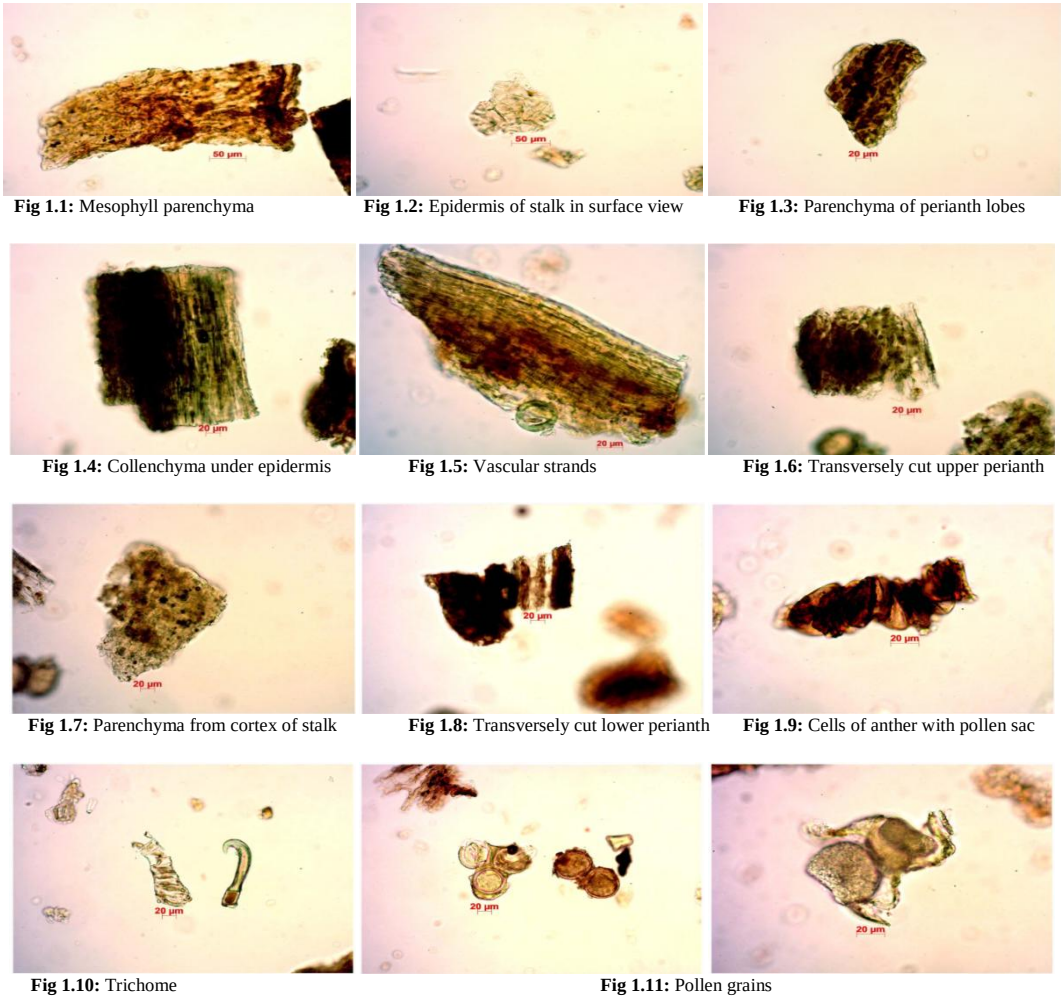


Fig 1.1: Mesophyll parenchyma
Fig 1.2: Epidermis of stalk in surface view
Fig 1.3: Parenchyma of perianth lobes
Fig 1.4: Collenchyma under epidermis
Fig 1.5: Vascular strands
Fig 1.6: Transversely cut upper perianth
Fig 1.7: Parenchyma from cortex of stalk
Fig 1.8: Transversely cut lower perianth
Fig 1.9: Cells of anther with pollen sac
Fig 1.10: Trichome
Fig 1.11: Pollen grains

Figure 1: Powder microscopy of *Ishwari* flower

Preliminary phytochemical analysis

Phytochemical analysis was done for presence of alkaloids, steroids, carbohydrates, tannin, flavanoids, saponins, triterpenoids, coumarins, phenols, carboxylic acid, resins, quinone and amino acid. The positive test got for steroids, carbohydrates, flavonoids, saponins and negative for alkaloids, tannin, triterpenoids, coumarins, phenols, carboxylic acid, resins, quinone and amino acid (Table 1).

Table 1: Results of preliminary phytochemical tests for *Aristolochia indica* Linn

Tests	Colour if positive	Colour observed	Result
Alkaloids			
Dragendrof's test	Orange precipitate	Orange color	–
Wagners test	Red precipitate	Red color	
Mayers test	Dull white precipitate	Colorless solution	
Hagers test	Yellow precipitate	Colorless solution	
Steroids			
Liebermann- Burchard test	Bluish green	Bluish green	+
Salkowski test	Bluish red to cherry red	Bluish red to cherry red	
Carbohydrate			
Molish test	Violet ring	Violet ring	+
Fehlings test	Brick red precipitate	Brick red precipitate	
Benedicts test	Red precipitate	Red precipitate	
Tannin			
With $FeCl_3$	Dark blue or green or brown	Yellow color	–
Flavanoids			+
Shinoda's test	Red to pink	Orange color	
Saponins			+
With $NaHCO_3$	Stable froth	Stable froth	
Triterpenoids			
Tin and thionyl chloride test	Pink	No pink	–
Coumarins			
With 2 N NaOH	Yellow	Yellow	–
Phenols			
With alcoholic ferric chloride	Blue to blue black, brown	Color of ferric chloride	–
Carboxylic acid			
With $NaHCO_3$	Brisk effervescence	No effervescence	–
Resin			
With aqueous acetone	Turbidity	No turbidity	–
Quinone			
5% NaOH	Pink/purple/red	Greenish yellow color	–
Amino acids			
Ninhydrine reagent	Purple color	Greenish color	–

- - Present; + - absent

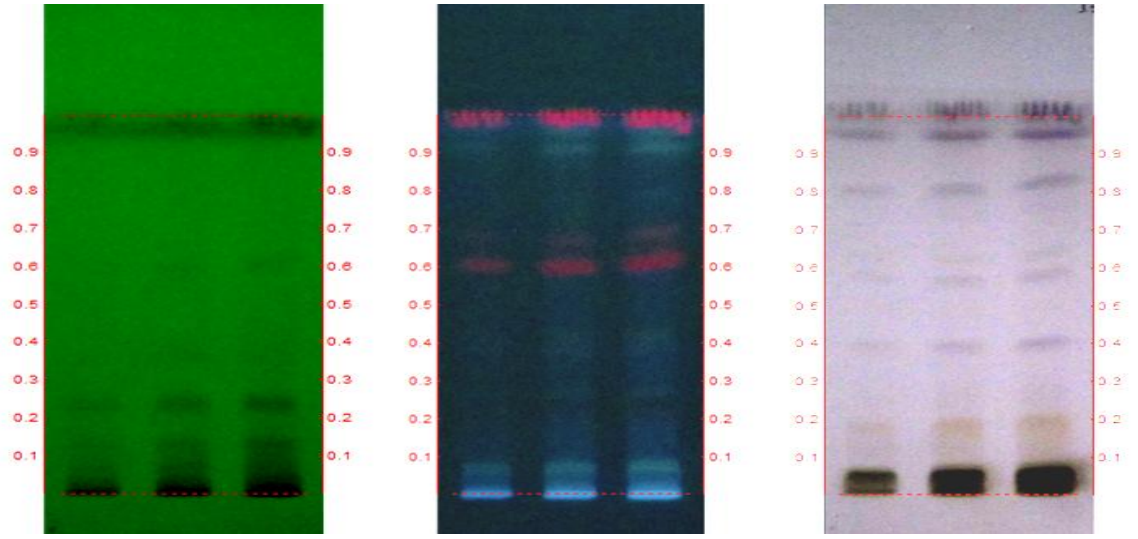
HPTLC

HPTLC of ethanol extract was carried out using toluene: ethyl acetate (8.0:1.0) as mobile phase for root; the $R_f$values and colour of the spots were recorded (Table 2). TLC photo-documentation revealed presence of many phytoconstituents with different R_f values and HPTLC densitometric scan of the plates showed numerous bands under 254 nm, 366 nm and 620 nm (after derivatisation). On photodocumentation there were 6 spots under 254 nm, 9 spots under 366 nm and 6 spots under 620 nm post-derivatisation with vanillin sulphuric acid spray reagent (Figure 2 and Table 2). Densitometrc scan at 254 nm revealed 9 peaks corresponding to 9 different compounds in the ethyl alcohol extract, compounds with R_f0.03 (49.53%) isthe major peaks (Figure 3). Densitometric scan at 366 nm showed 7 peaks, peak with R_f0.02 (28.71%) & 0.69 (46.77%) were the major peaks detected (Figure 4).

Table 2: R_f values of samples

At 254m	At 366m	Post derivatisation
0.08 (L. green)	0.08 (FL. green)	-
0.13 (L. green)	-	-
-	-	0.20 (L. orange)
0.25 (D. green)	-	-
-	0.28 (FL.green)	-
0.38 (L. green)	0.38 (FL. blue)	-
-	-	0.40 (D. purple)
-	0.43 (FL. green)	-
-	-	0.57 (D. purple)
0.60 (L. green)	0.60 (FD. red)	-
-	-	0.63 (L. purple)
0.67 (L. green)	-	-
-	0.69 (FL. red)	-
-	-	0.78 (L. purple)
-	0.82 (FL. green)	0.82 (D. purple)
-	0.92 (FL. green)	-
-	0.96 (FL. green)	-

*L-light; D-Dark; F-Fluorescent

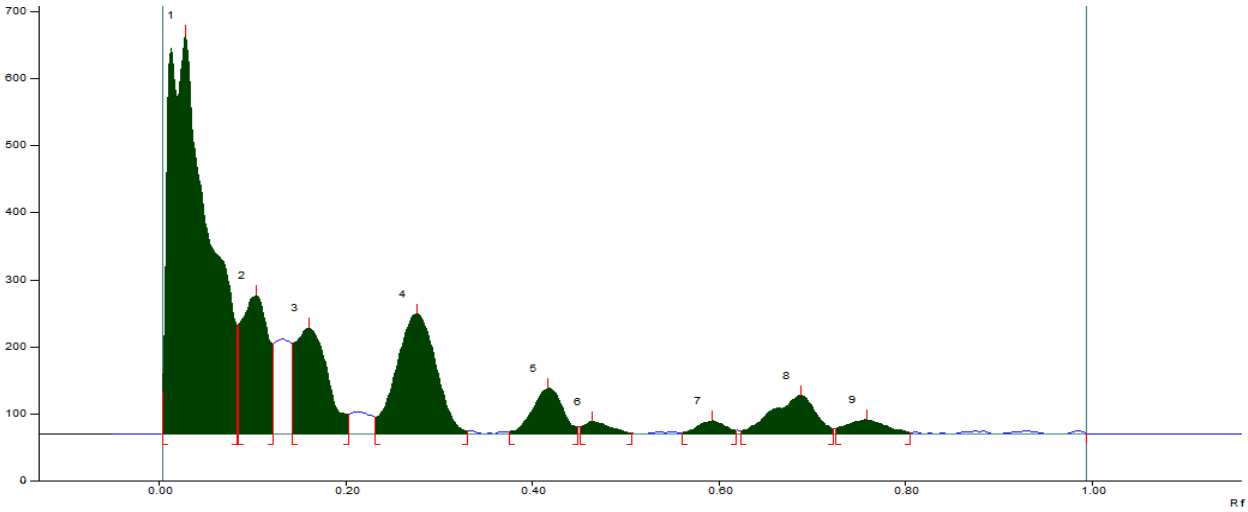


At 254m At 366m Post derivatisation

Figure 2: HPTLC Photo documentation of alcoholic extract of *Ishwari* flower

Track 1- 3µl; Track 2- 6µl; Track 3- 9µl

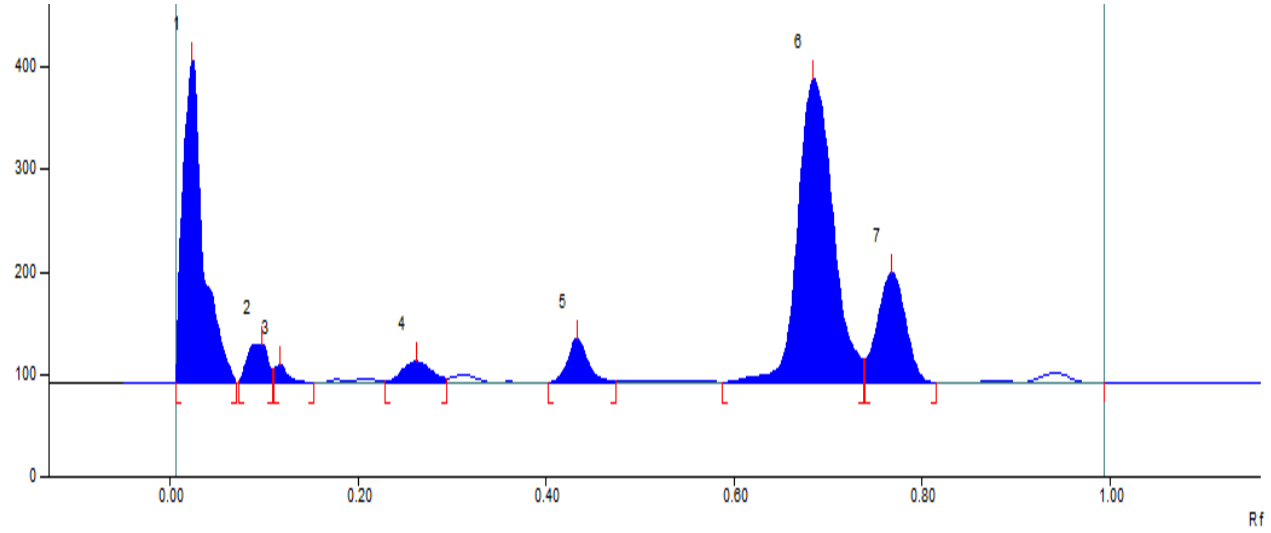
Solvent system: Toluene: Ethyl acetate (8.0: 1.0)



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	61.6 AU	0.03 Rf	593.9 AU	44.88 %	0.08 Rf	60.5 AU	18201.2 AU	49.53 %
2	0.09 Rf	161.1 AU	0.10 Rf	206.3 AU	15.59 %	0.12 Rf	34.1 AU	4255.0 AU	11.58 %
3	0.14 Rf	135.8 AU	0.16 Rf	158.2 AU	11.95 %	0.20 Rf	29.7 AU	3968.8 AU	10.80 %
4	0.23 Rf	25.4 AU	0.28 Rf	179.2 AU	13.54 %	0.33 Rf	5.1 AU	5422.6 AU	14.75 %
5	0.38 Rf	3.8 AU	0.42 Rf	68.5 AU	5.18 %	0.45 Rf	10.6 AU	1559.9 AU	4.24 %
6	0.45 Rf	10.7 AU	0.46 Rf	18.8 AU	1.42 %	0.51 Rf	0.8 AU	380.7 AU	1.04 %
7	0.56 Rf	2.3 AU	0.59 Rf	20.0 AU	1.51 %	0.62 Rf	5.4 AU	420.1 AU	1.14 %
8	0.62 Rf	5.3 AU	0.69 Rf	57.3 AU	4.33 %	0.72 Rf	8.0 AU	1895.8 AU	5.16 %
9	0.73 Rf	8.2 AU	0.76 Rf	21.1 AU	1.59 %	0.81 Rf	2.1 AU	647.0 AU	1.76 %

Ishwari (9µl)at 254 nm

Figure 3: Densitometric scan of *Ishwari*Flower



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.0 AU	0.02 Rf	313.5 AU	37.50 %	0.07 Rf	0.8 AU	4909.3 AU	28.71 %
2	0.07 Rf	1.0 AU	0.10 Rf	37.9 AU	4.53 %	0.11 Rf	13.2 AU	589.8 AU	3.45 %
3	0.11 Rf	13.4 AU	0.12 Rf	18.0 AU	2.15 %	0.15 Rf	0.0 AU	175.6 AU	1.03 %
4	0.23 Rf	1.5 AU	0.26 Rf	20.7 AU	2.47 %	0.30 Rf	4.5 AU	473.6 AU	2.77 %
5	0.40 Rf	0.4 AU	0.43 Rf	43.3 AU	5.18 %	0.48 Rf	1.8 AU	670.6 AU	3.92 %
6	0.59 Rf	0.6 AU	0.69 Rf	295.5 AU	35.34 %	0.74 Rf	22.7 AU	7995.8 AU	46.77 %
7	0.74 Rf	22.8 AU	0.77 Rf	107.3 AU	12.83 %	0.82 Rf	0.3 AU	2283.1 AU	13.35 %

Ishwari(9µl)at 366 nm

Figure 4: Densitometric scan of *Ishwari*at 9 µl

Acute toxicity study

The animal was observed for physical and behavioral changescontinuously for 4 hours after the dosing. The careful cage side observation was done without disturbing the animal attention and at the end of the every hour the animal was individually exposed to open arena for recording the behavioral changes like increased or decreased motor activity, convulsions, straub's reaction, muscle spasm, catatonia, spasticity, ophisthotonus, hyperesthesia, muscle relaxation, anesthesia, arching and rolling, lacrimation, salivation, diarrhea, writhing, mode of respiration, changes in skin colour, exitus, CNS depression – hypo activity, passivity, relaxation, ataxia, narcosis, etc. There were no physical and behavioral changes-except mild increase in motor activity, irritability, and rearing activity seen in 2 rats in the group 2000 mg/kg in all the treated animals on day one at 1, 2, 3 and 4 hours intervals after dosing and there after once daily for 14 consecutive days. Thus the data obtained from the study on single dose administration of coded drug flower of*Ishwari*oral administration up to 14 days of observation period does not result in any physical and behavioral changes.

Animals were kept under observation for mortality if any at ½, 1, 2, 3, 4, 24 h, 48 h after dosing, and, there after daily once during the entire period of the study (i.e.14 days). All the animals belonging to the treated group survived throughout the 14 days observation period after dosing.

Conclusion

The given samples have been tested as per standard testing protocol. The flower powder microscopical characters were examined and reported. The extract was tested positive for steroids, carbohydrates, flavonoids, saponins. HPTLC photo documentation, R_f values and densitometric scan at 254 nm, 366 nm and after derivatisation has been developed and reported. Thin layer chromatography has shown many compounds in 366 nm frequency. Acute oral toxicity test (AOT) proved flower to be nontoxic. Flowers are much safer to use orally as it is found to be nontoxic and 1/10th of the dose, i.e. 200 mg/kg is proposed as therapeutic dose for further pharmacological evaluation of flowers. The results obtained from the study can be used for evaluation of the flower of *Aristolochia indica* Linn as substitute for roots.

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Conflict of interest

None declared.

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