Journal of Scientific and Innovative Research 2015; 4(2): 94-99
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2015; 4(2): 94-99

Received: 02-03-2015
Accepted: 05-05-2015

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Analytical standards for root and leaf of *Ishwari- Aristolochia indica* Linn.

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Abstract

Analytical standard is a numerical value or specific property that quantifies the purity and quality of drug and formulated medicine. By standardizing raw drugs, methods, and formulations we can provide standard parameters to assess the quality, safety and efficacy of medicines. Such evaluation of a crude drug is necessary for assessing phytochemical variations, deterioration, substitution, and adulteration. Ayurvedic drug *Ishwari's* botanical source is *Aristolochia indica* Linn. The synonym *Nakuli* has been used instead of *Ishwari* in *Samhithas*. The drug has been mentioned in different form for the condition like *sheethajwara*, *sarpavisha*, *vrana*, *ekangashopha*, *unmada*, *apasmara* etc. In the current study, analytical standards for roots and leaves of *Ishwari* were done as per Pharmacopoeial procedures. Analytical values are comparatively higher in leaves than root. Ethanol extractive of both root and leaves are lesser compared to aqueous extractive. Preliminary phytochemical tests of both the parts have shown similar results for the presence of alkaloid, steroid, tannins, triterpenoids, coumarin, phenols, carboxylic acid and absence of carbohydrates, saponin, flavanoids and resins. Thin layer chromatography has show more compounds in 366 nm both in root as well as in leaf. The standards obtained can be used as analytical values for routine standardization of *Ishwari* root and leaf.

Keywords: Aristolochiaceae, HPTLC, *Nakuli*, Physicochemical, phytochemical.

Introduction

The demand for Ayurvedic medicine as well as other natural products for health care is increasing globally. Their acceptibility and future prospectus are associated wth the quality standards of these products. In the changing scenario of advanced civilization, ecological variations, pollutions and advent of newer technologies, the present laboratory based parameters are necessary for standardization of herbal drugs for its identity, purity and strength. As global market for Ayurvedic medicinal products is increasing tremendously, need for universally acceptable standardization parameters is felt by the authorities like Ayurvedic pharmacopoeia of India, Ayurvedic formulary of India, Indian pharmacopoeia and so on. Standardization means derivation of a numerical value or specific property that quantifies the purity and quality of drug and formulated medicine. By standardizing the raw drugs, methods and formulations we can provide standard parameters to assess the quality, safety and efficacy of medicines[1]. The drug *Ishwari*has its source as *Aristolochia indica* Linn. and *Nakuli*as synonym in *Samhithas*. In *Carakasamhita* the drug has been mentioned in the preparation of *taila* for *sheethajwara* and in the preparation of *ghrita* for *Jwara, unmade & apasmara*[2]. In *Sushrutasamhita* it has been mentioned in the preparation of lepa for *sarpa visha*[3]. *Acharya Vagbhata* used this drug as *lepa* for *ekangashopha* and preparation of oil for *sheetajajwara*[4]. *Guna Karma* of the drug is mentioned in *hareetakyaadivarga* of *Bhavaprakasha* where the drug is said to be effective in wound healing process[5]. The plant root is said to be usedmainly in fever, children's bowel complaint and specially root and leaf in the management of snake bite poisoning[6].

Uniformity of quality is promoted by the use of standards which are generally numerical quantities by which the quality and purity of the drug may be assessed. In this study standardization of root and leaf of *Aristolochia indica* Linn.was performedby performingphysico chemical, preliminary phytochemical and HPTLCanalysis.

Material and Methods

Materials

The authentic samples of dry root and leaf of *A. indica*was collected inand around Udupi district of Karnataka, India. It was identified and authenticated by comparison with the botanical description mentioned in Flora[7]. The root and leaf are shade dried and the voucher specimen (No. 255/13051008) was deposited at the Pharmacognosy Laboratory of SDM Centre for Research in Ayurveda & Allied Sciences, Udupi for future reference. Coarse powder of the materials was used for the analyses.

Methods

Physico-chemical evaluation

Foreign matter, total ash, acid insoluble ash, loss on drying at 105^{o}C (LOD), ethanol soluble extractive and water soluble extractivewas performed as per standard protocol[8,9]. Preliminary phytochemical investigation was done to detect the presence of alkaloids, steroids, carbohydrates, tannin, flavanoids, saponins, triterpenoids, coumarins, phenols, resins and carboxylic acidin ethanol extract[10].

One gram of the powders was extracted exhaustively with 10 ml of ethanol using Soxhlet extractor, the extract is made up to 10 ml. Five and 10 micro litre of the above was applied using CAMAG Linomat[5] applicator, separation was obtained using toluene: ethyl acetate: methanol (5:4:1) for rootand toluene: ethyl acetate (1:1) for leaf as mobile phase on silica gel G F254pre-coated on aluminium sheets of 0.2 mm thickness[11,12]. The $R_f$values were determined from the photo-documentation performed using CAMAG photo-documentation cabinet and the plates were scanned under 254 nm, 366 nm and 620 nm after derivatisationusing CAMAG Scanner[4].

Result and Discussion

Physico-chemical tests

The root and leaf of the plant is studied for its physicochemical parameters like foreign matter,total ash,acid insoluble ash, loss on drying,ethanol soluble extractive and water soluble extractive. The foreign matterwas nil in both root and leaf. The total ash indicating total inorganic content was found to be 2.84 and 12.45 in root and leaf respectively. Acid insoluble part of total ash, which indicates silica, was found to be 0.3 and 0.7 respectively. Loss on drying indicating moisture and other volatile matter was determined to be 11.75 and 13.77 %. Ethanol and water soluble secondary metabolites in in root and leaf was found to be 3.63, 9.26 and 9.6, 2.54 % w/w respectively. The result has shown significant difference in physico-chemical constants of root and leaf. Total ash and acid insoluble ash are more in leaf compare to root indicating presence of high salt and silica in leaf. LOD is almost 2% more in leaf when compare to root.Both ethanol and water soluble extract is more in leaf when compared to root.Among leaf and root water soluble extract is more than ethanol extractive (Table 1).

Table 1: Physico-chemical parameters of Iswarileaf and root

Parameters	**Results n=3 % w/w**	
	Root	**Leaf**
Foreign matter	Nil	Nil
Total ash	2.84	12.45
Acid insoluble ash	0.30	0.75
Loss on drying	11.75	13.77
Ethanol soluble extractive	3.63	9.6
Water soluble extractive	9.26	20.54

Preliminaryphytochemical tests

Root and leaf showed presence of alkaloid, steroid, tannin, triterpenoid, carboxylic acid, coumarin and phenols;carbohydrate, flavanoid, saponins and resin was absent. There is no difference in presence of chemicals present in root and leaf, but dragendrofs test for alkaloid in leaf was negative whereas same was positive in root (Table 2).

Table 2: Results of preliminary phytochemical tests of root and leaf

Tests	**Colour if positive**	***Iswari***			
		Root	**Inference**	**Leaf**	**Inference**
Alkaloids					
Dragendrof's test	Orange precipitate	Orange	+	Brown	-
Wagners test	Red precipitate	Red precipitate	+	Red precipitate	+
Mayers test	Dull white precipitate	Light white precipitate	+	Light white colour	+
Hagers test	Yellow precipitate	Yellow colour	+	Yellow colour	+
Steroids					
Liebermann- Buchard test	Bluish green	Greenish brown colour	+	Green colour	+
Salkowski test	Bluish red to cherry red	Red colour	+	Light red colour	+
Carbohydrate					
Molish test	Violet ring	Brown colour	-	Brown colour	-
Fehlings test	Brick red precipitate	Blue colour	-	Blue colour	-
Benedicts test	Red precipitate	Blue colour	-	Blue colour	-
Tannin					
With $FeCl_3$	Dark blue or green or brown	Light brown colour	+	Light brown colour	+
Flavanoids					
Shinoda's test	Red to pink	Light brown colour	-	Light green colour	-
Saponins					
With distilled water	Stable froth	No froth	-	No froth	-
Triterpenoids					
Tin and thionyl chloride test	Pink	Very light pink colour	+	Very light pink colour	+
Coumarins					

With 2 N NaOH	Yellow	Yellow colour	+	Yellow colour	+
Phenols					
With ethanolicferric chloride	Blue to blue black, brown or green	Light brown colour	+	Light brown colour	+
Resins					
With acetone and distilled water	Turbidity	No turbidity	-	No turbidity	-
Carboxylic acid					
With $NaHCO_3$	Effervescence	Little effervescence	+	Little effervescence	+

HPTLC

R_f values and colour of the spots in chromatogram developed intoluene: ethyl acetate: methanol (5:4:1) for ethanolic extract of root was recorded (Table 3). 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). Onphotodocumentation there were 3 spots under 254 nm, 10 spots under 366 nm and 10 spots under 620 nm post-derivatisation with vanillin sulphuric acid spray reagent (Figure 1 and Table 3). Densitometrc scan at 254 nm revealed 9 peaks corresponding to 9 different compounds in the ethanol extract, compounds with R_f 0.06 (19.41%), 0.26 (20.24%), and 0.61 (39.11%) werethe major peaks (Figure 3). Densitometric scan at 366 nm showed 11 peaks, peak with R_f 0.20 (13.57%), 0.29 (24.65%), 0.32 (14.25%), and 0.81 (23.17%) were the major peaks detected (Figure 4).

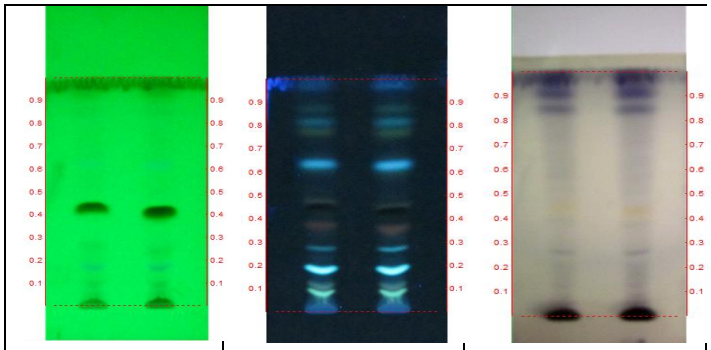
HPTLC of ethanol extract of leaf was carried out usingtoluene: ethyl acetate (1:1) as mobile phase; the $R_f$values and colour of the spots were recorded (Table 4). TLC photo-documentation revealed presence of many phytoconstituents with different R_f values and HPTLC densitometric scan of the plates showed numerous bands under 254, 366 and 620 nm after derivatisation. On photodocumentation there were 3 spots under 254 nm, 7 spots under 366 nm, and 6 spots under 620 nm post-derivatisation with vanillin sulphuric acid spray reagent (Table 4 and Figure 2). Densitometrc scan at 254 nm revealed 9 peaks corresponding to 9 different compounds in the ethanol extract, compounds with R_f 0.04 (28.90%), 0.05 (35.81%), and 0.90 (14.49%) werethe major peaks (Figure 5). Densitometric scan at UV 366 nm showed 10 peaks, peak with R_f 0.30 (19.40%), and 0.50 (29.48%), were the major peaks detected (Figure 6).

Table 3: R_f values of all tracks (root)

At 254 nm	At 366 nm	Post derivatisation
-	-	0.05 Violet
-	0.07 F green	0.07 L violet
0.10 Green	0.10 F L Blue	-
-	-	-
-	0.17 F blue	-
0.23 L green	-	-
-	0.27 F blue	0.27 Violet
-	0.37 F L red	0.37L green
0.41 D Green	-	-
-	-	0.43 Yellow
-	0.46 F black	-
-	0.48 F yellow	-
-	-	0.51 L blue
-	-	0.56 L violet
-	-	0.62 L violet
-	0.64 F blue	-
-	-	0.71 L violet
-	0.77 F green	-
-	0.81F blue	-
-	-	0.84 Violet
-	0.87 F green	-
-	-	0.91 Violet

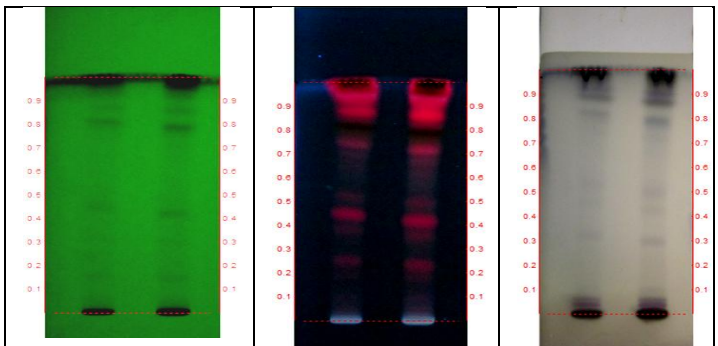
Table 4: R_f values of all tracks (leaf)

At 254 nm	At 366 nm	Post derivatisation
-	-	0.04 Violet
-	-	0.06 D blue
-	0.09 F red	-
0.15 L green	-	-
-	0.23 F red	-
0.30 L green	-	0.30 L violet
-	0.36 F red	-
0.42 Green	0.42 F red	0.42 L violet
-	0.49 F red	0.49 L violet
-	0.67 F red	-
-	0.71 F red	0.71 L violet
0.79 D green	0.79 F black	0.79 Blue
0.86 Green	0.86 F red	0.86 Violet
-	-	0.91 Violet



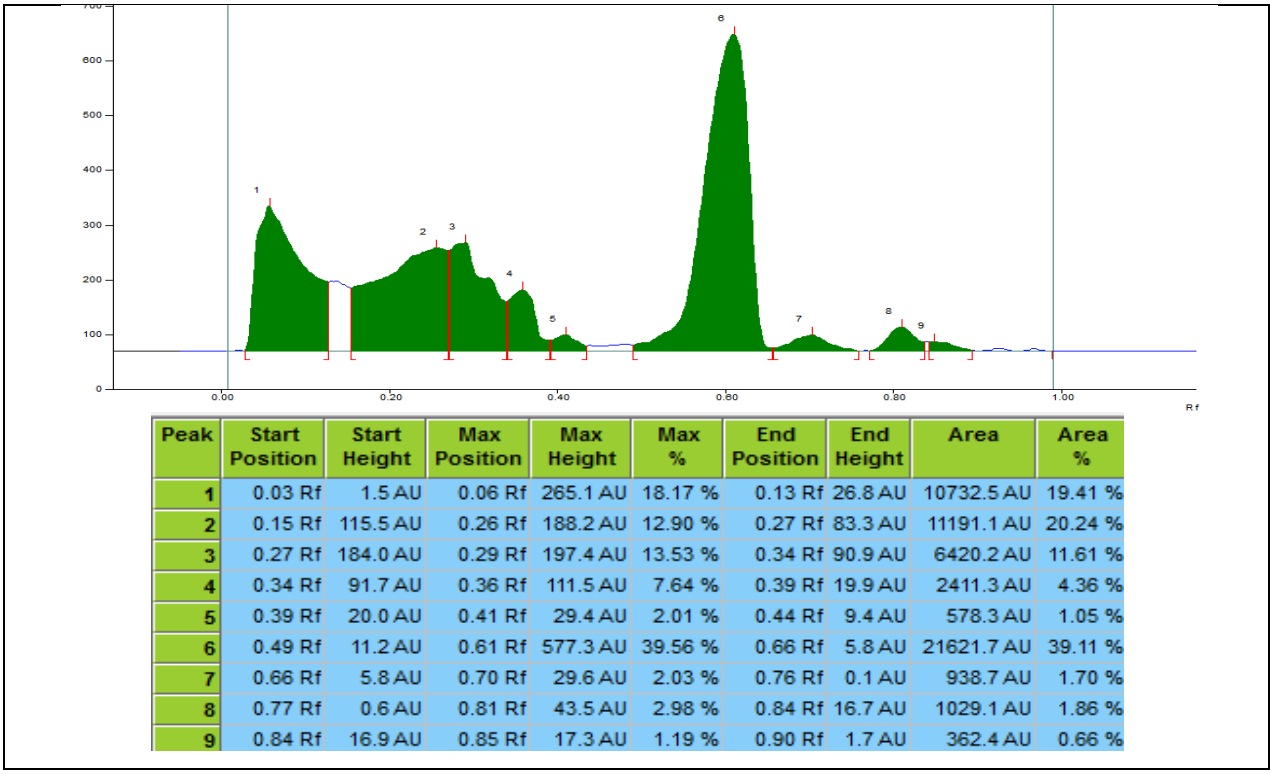
Solvent system: Toluene: Ethyl acetate: Methanol (5: 4: 1)

Figure 1: TLC photodoccumentation of *Iswari* root



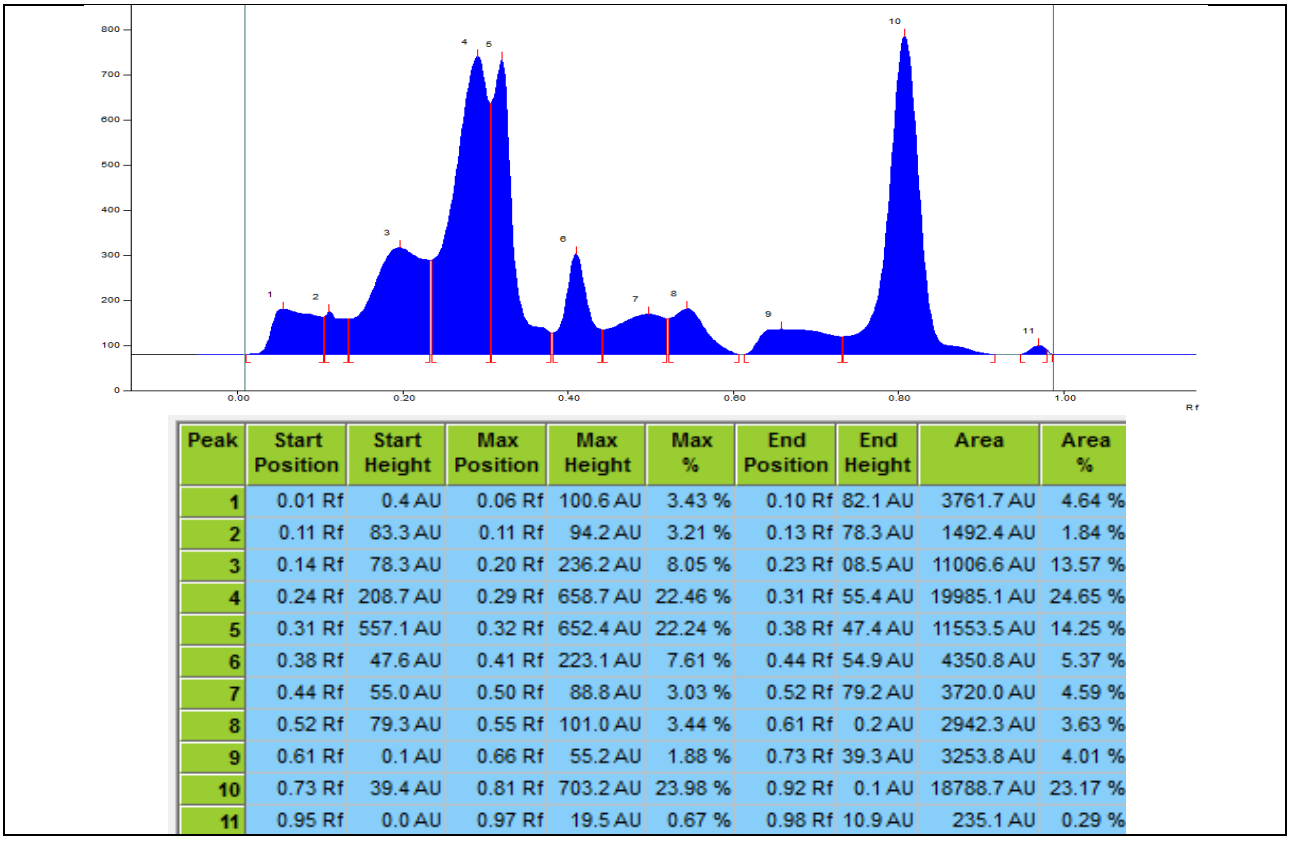
Solvent system: Toluene: Ethyl acetate (1: 1)

Figure 2: TLC photodoccumentation of *Iswari* leaf



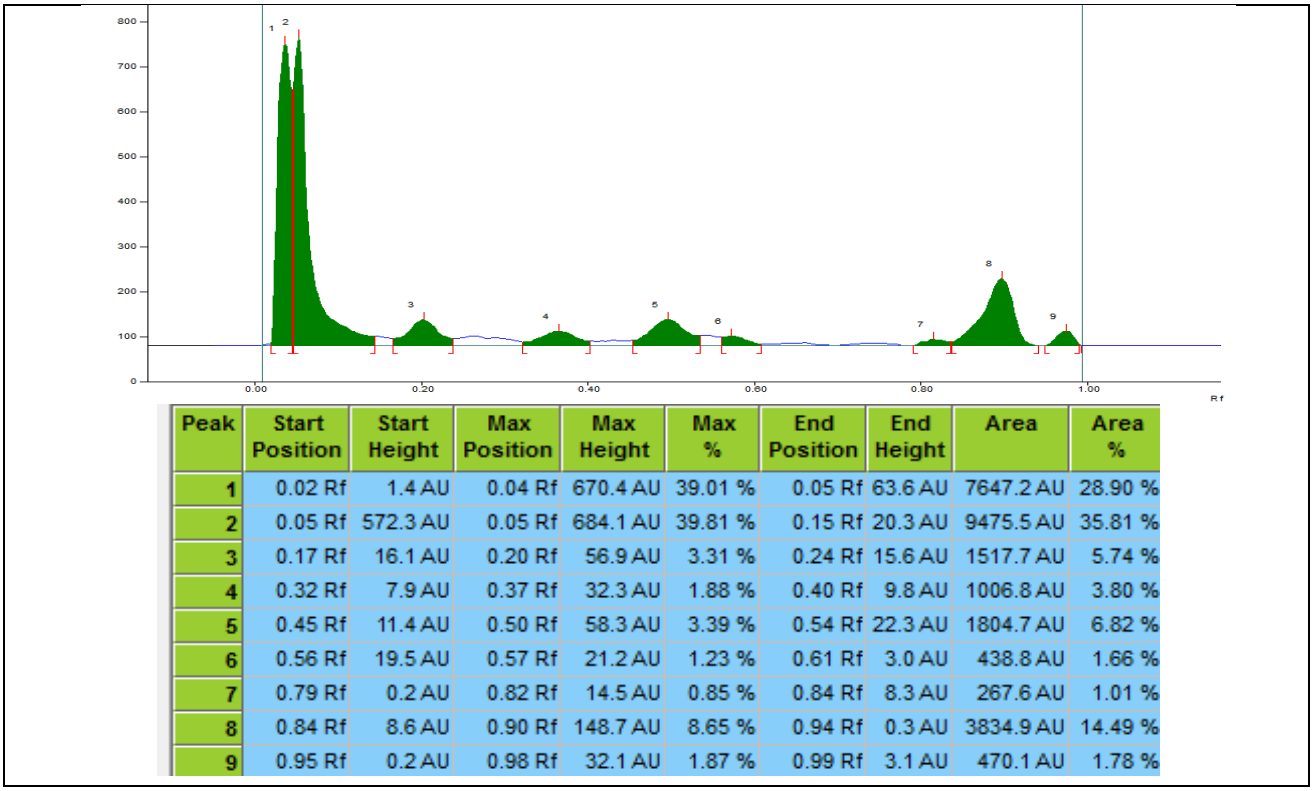
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	1.5 AU	0.06 Rf	265.1 AU	18.17 %	0.13 Rf	26.8 AU	10732.5 AU	19.41 %
2	0.15 Rf	115.5 AU	0.26 Rf	188.2 AU	12.90 %	0.27 Rf	83.3 AU	11191.1 AU	20.24 %
3	0.27 Rf	184.0 AU	0.29 Rf	197.4 AU	13.53 %	0.34 Rf	90.9 AU	6420.2 AU	11.61 %
4	0.34 Rf	91.7 AU	0.36 Rf	111.5 AU	7.64 %	0.39 Rf	19.9 AU	2411.3 AU	4.36 %
5	0.39 Rf	20.0 AU	0.41 Rf	29.4 AU	2.01 %	0.44 Rf	9.4 AU	578.3 AU	1.05 %
6	0.49 Rf	11.2 AU	0.61 Rf	577.3 AU	39.56 %	0.66 Rf	5.8 AU	21621.7 AU	39.11 %
7	0.66 Rf	5.8 AU	0.70 Rf	29.6 AU	2.03 %	0.76 Rf	0.1 AU	938.7 AU	1.70 %
8	0.77 Rf	0.6 AU	0.81 Rf	43.5 AU	2.98 %	0.84 Rf	16.7 AU	1029.1 AU	1.86 %
9	0.84 Rf	16.9 AU	0.85 Rf	17.3 AU	1.19 %	0.90 Rf	1.7 AU	362.4 AU	0.66 %

Figure 3: HPTLC photodoccumentation of *Iswari* root at 254 nm



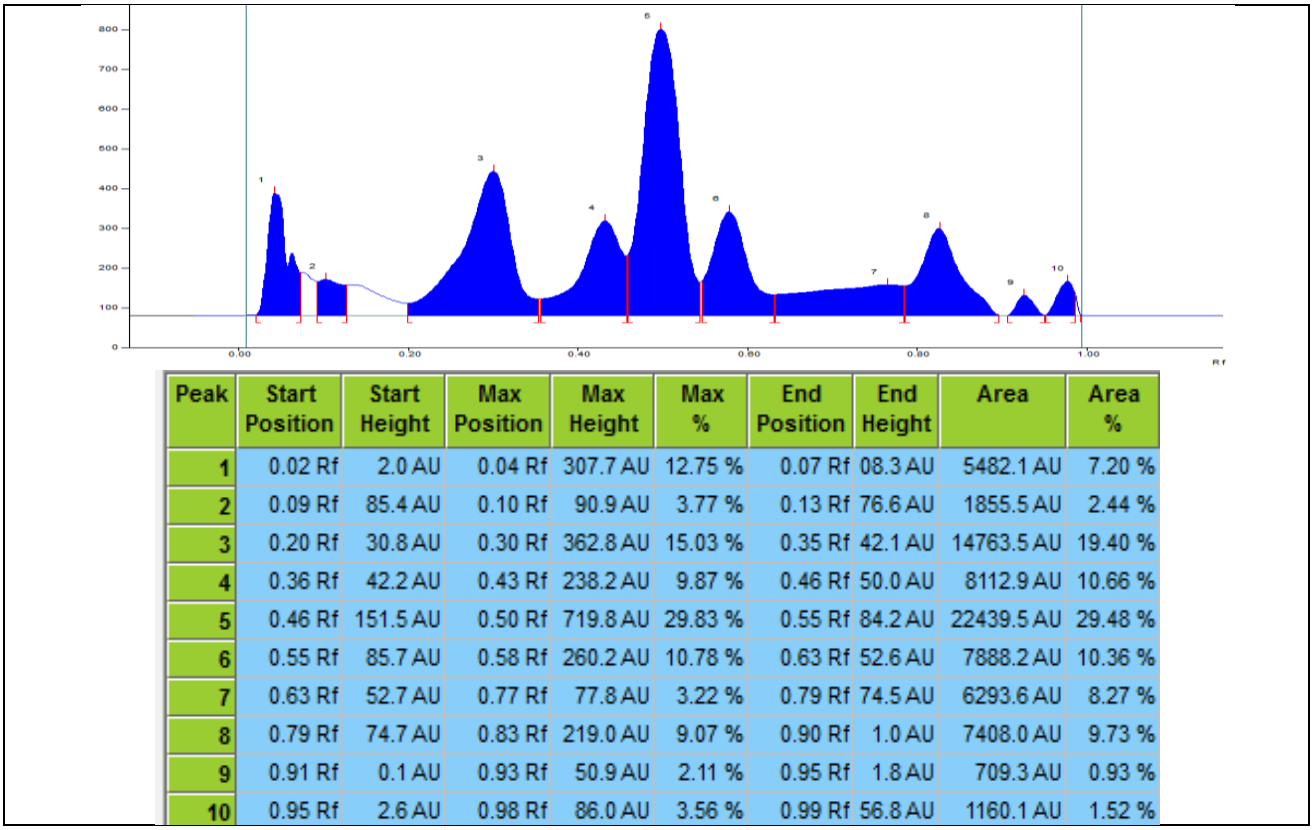
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.4 AU	0.06 Rf	100.6 AU	3.43 %	0.10 Rf	82.1 AU	3761.7 AU	4.64 %
2	0.11 Rf	83.3 AU	0.11 Rf	94.2 AU	3.21 %	0.13 Rf	78.3 AU	1492.4 AU	1.84 %
3	0.14 Rf	78.3 AU	0.20 Rf	236.2 AU	8.05 %	0.23 Rf	08.5 AU	11006.6 AU	13.57 %
4	0.24 Rf	208.7 AU	0.29 Rf	658.7 AU	22.46 %	0.31 Rf	55.4 AU	19985.1 AU	24.65 %
5	0.31 Rf	557.1 AU	0.32 Rf	652.4 AU	22.24 %	0.38 Rf	47.4 AU	11553.5 AU	14.25 %
6	0.38 Rf	47.6 AU	0.41 Rf	223.1 AU	7.61 %	0.44 Rf	54.9 AU	4350.8 AU	5.37 %
7	0.44 Rf	55.0 AU	0.50 Rf	88.8 AU	3.03 %	0.52 Rf	79.2 AU	3720.0 AU	4.59 %
8	0.52 Rf	79.3 AU	0.55 Rf	101.0 AU	3.44 %	0.61 Rf	0.2 AU	2942.3 AU	3.63 %
9	0.61 Rf	0.1 AU	0.66 Rf	55.2 AU	1.88 %	0.73 Rf	39.3 AU	3253.8 AU	4.01 %
10	0.73 Rf	39.4 AU	0.81 Rf	703.2 AU	23.98 %	0.92 Rf	0.1 AU	18788.7 AU	23.17 %
11	0.95 Rf	0.0 AU	0.97 Rf	19.5 AU	0.67 %	0.98 Rf	10.9 AU	235.1 AU	0.29 %

Figure 4: HPTLC photodoccumentation of *Iswari* root at 366 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	1.4 AU	0.04 Rf	670.4 AU	39.01 %	0.05 Rf	63.6 AU	7647.2 AU	28.90 %
2	0.05 Rf	572.3 AU	0.05 Rf	684.1 AU	39.81 %	0.15 Rf	20.3 AU	9475.5 AU	35.81 %
3	0.17 Rf	16.1 AU	0.20 Rf	56.9 AU	3.31 %	0.24 Rf	15.6 AU	1517.7 AU	5.74 %
4	0.32 Rf	7.9 AU	0.37 Rf	32.3 AU	1.88 %	0.40 Rf	9.8 AU	1006.8 AU	3.80 %
5	0.45 Rf	11.4 AU	0.50 Rf	58.3 AU	3.39 %	0.54 Rf	22.3 AU	1804.7 AU	6.82 %
6	0.56 Rf	19.5 AU	0.57 Rf	21.2 AU	1.23 %	0.61 Rf	3.0 AU	438.8 AU	1.66 %
7	0.79 Rf	0.2 AU	0.82 Rf	14.5 AU	0.85 %	0.84 Rf	8.3 AU	267.6 AU	1.01 %
8	0.84 Rf	8.6 AU	0.90 Rf	148.7 AU	8.65 %	0.94 Rf	0.3 AU	3834.9 AU	14.49 %
9	0.95 Rf	0.2 AU	0.98 Rf	32.1 AU	1.87 %	0.99 Rf	3.1 AU	470.1 AU	1.78 %

Figure 5: HPTLC photodoccumentation of *Iswari* leaf at 254 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	2.0 AU	0.04 Rf	307.7 AU	12.75 %	0.07 Rf	08.3 AU	5482.1 AU	7.20 %
2	0.09 Rf	85.4 AU	0.10 Rf	90.9 AU	3.77 %	0.13 Rf	76.6 AU	1855.5 AU	2.44 %
3	0.20 Rf	30.8 AU	0.30 Rf	362.8 AU	15.03 %	0.35 Rf	42.1 AU	14763.5 AU	19.40 %
4	0.36 Rf	42.2 AU	0.43 Rf	238.2 AU	9.87 %	0.46 Rf	50.0 AU	8112.9 AU	10.66 %
5	0.46 Rf	151.5 AU	0.50 Rf	719.8 AU	29.83 %	0.55 Rf	84.2 AU	22439.5 AU	29.48 %
6	0.55 Rf	85.7 AU	0.58 Rf	260.2 AU	10.78 %	0.63 Rf	52.6 AU	7888.2 AU	10.36 %
7	0.63 Rf	52.7 AU	0.77 Rf	77.8 AU	3.22 %	0.79 Rf	74.5 AU	6293.6 AU	8.27 %
8	0.79 Rf	74.7 AU	0.83 Rf	219.0 AU	9.07 %	0.90 Rf	1.0 AU	7408.0 AU	9.73 %
9	0.91 Rf	0.1 AU	0.93 Rf	50.9 AU	2.11 %	0.95 Rf	1.8 AU	709.3 AU	0.93 %
10	0.95 Rf	2.6 AU	0.98 Rf	86.0 AU	3.56 %	0.99 Rf	56.8 AU	1160.1 AU	1.52 %

Figure 6: HPTLC photodoccumentation of *Iswari* leaf at 366 nm

Conclusion

Root and leaves of *Ishwari* have been standardized as per pharmacopoeial testing protocol. The results of physico-chemical parameters and preliminary phytochemical test have been reported. HPTLC photo documentation, R_f values and densitometric scan at 254 nm, 366 nm and after derivatisation has been developed.The physico-chemical constants of leaf are comparatively higher than root. Ethanol extractive of both root and leaves are lesser when compared to aqueous extractive. Phytochemical testing of the drugs shown similar results. The test was positive for alkaloid, steroid, tannins, triterpenoids, coumarin, phenols, carboxylic acid and negative for carbohydrates, saponin, flavanoids and resins. Thin layer chromatography has shownmore compounds in 366 nm frequency both in root as well as in leaf. Results obtained from the study can be used for analyticalstandardization of the drug *Aristolochia indica* Linn.

Acknowledgement

Authors are grateful to revered President, Dr. D. Veerendra Heggade and Dr. B. Yashovarma, Secretary, SDM Educational Society for encouragement. Guidance by Dr. K. R.Ramachandra, Principal SDM College of Ayurveda, Kuthpady, Udupi is gratefully acknowledged. Author areindebted to Dr. B. Ravishankar, Director; SDM Centre for Research in Ayurveda and Allied Sciences, Udupi for providing facilities.

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