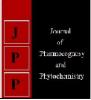


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#### Smita Bhat

PG Department of Studies in Botany, Karnatak University, Dharwad, Karnatak, India

#### GS Mulgund

PG Department of Studies in Botany, Karnatak University, Dharwad, Karnatak, India

#### Shruti Hegde

PG Department of Studies in Botany, Karnatak University, Dharwad, Karnatak, India

#### Harsha V Hegde

National Institute of Traditional Medicine, ICMR, Belagavi, Karnataka, India

#### Correspondence Smita Bhat PG Department of Studies in Botany, Karnatak University, Dharwad, Karnatak, India

# Pharmacognostic evaluation and phytochemical analysis of leaf and tuber of *Ariopsis peltata* Nimmo.

# Smita Bhat, GS Mulgund, Shruti Hegde and Harsha V Hegde

#### Abstract

*Ariopsis peltata* Nimmo. (Araceae) is a tuberous, perennial herb distributed along the Western Ghats. In the present study, an attempt was made to evaluate pharmacological and preliminary phytochemical screening of different extracts of plant. The phytochemical analysis and TLC profiling was carried out and perceived the presence of carbohydrates, alkaloids, fats and oils, proteins, amino acid, steroids, glycosides and phenolic compounds. Powder microscopic analysis have shown the presence of starch grains, epidermal cells, libri form fiber, cluster and needle shaped crystal, pitted parenchyma and xylem elements such as reticulate and spiral vessels. TLC profiling of plant extract shown different fluorescent phytochemical compounds with different  $R_f$  values of which 0.82 and 0.80 were clear spots observed in solvent combination of hexane: ethyl acetate (6:4) for leaf extract. However, the phytochemical compounds which are responsible for biological activity recorded from present investigation may possibly give a potent plant based natural drug.

Keywords: Ariopsis peltata; pharmacognosy; phytochemical analysis; powder microscopy; thin layer chromatography.

#### Introduction

All over the world, plants have served as richestbio-resourcefor traditional as well as modern medicinal system. In developing countries, because of easy availability and lesser side effect about 80% of the population totally depends on herbal medicine for their primary health care needs. According to an estimate of WHO approximately 25% of drugs are used today in modern medicine were derived from plants and plant derivatives <sup>[21]</sup>. Phytochemicals are the natural bioactive compounds which are derived from different plant parts, primarily responsible for medicinal activities. Recent finding suggest that majority of phytochemical have been known for valuable therapeutic activity such as anti- diabetic, anti-bacterial, anti-arthritic and anti-cancerous.

Lectins are carbohydrate-binding proteins of non-immune origin that agglutinates the complex carbohydrates. Lectins are distinguished from other carbohydrates because of recognizing specific binding site and attach reversibly to carbohydrates without altering their chemical structure. During last few decades an increasing number of lectins have been reported from various monocotyledonous plant species and only few lectins are characterized from Araceae members as compared with other families. Several works have been reported on the isolation aspect of monocot lectins from the family Araceae, viz. *Arisaema consanguineum, Arisaema curvatum, Sauromatum guttatum, Gonatanthus pumilus, Arum maculatum, Colocasia esculenta, Xanthosoma sagittifolium and Dieffenbachia sequina* <sup>[17,19,20]</sup>. Among these new monocot lectin reported from *Xanthosoma sagittifolium* showed significant inhibition results towards human and murine cancer cell- line<sup>[20,27]</sup>. However, plant lectins have shown antitumor and anti- carcinogenic activity like other drugs which inhibit apoptosis of tumor cells and also shown cytotoxic effect mediated by means of apoptosis <sup>[3, 20, 22]</sup>.

*Ariopsis peltata* a small, tuberous, perennial herb with peltate leaves, belonging to family Araceae. It is commonly known as "shield leaf ariopsis" and mature plant grown up to 15-25 cm long. It is distributed abundantly across Indian plains, Western Ghats and from Nepal to Sikkim <sup>[1, 2]</sup>. *Ariopsis peltata* is lithophytic herb growing in crevices of rock with tubers 1-3 cm in diameter. Leaves are shield shaped with 4-15 cm long and 3-9 cm wide lamina, petiole is 8-20 cm long, slender, where petiole joins lower central portion of leaf. Lamina peltate, cordate - ovate, thin, glaucous below with short posterior lobes. The species name peltata highlights the structure of lamina. Venation is reticulate, marginal veins are present, primary lateral veins are pinnate, radiating from the petiole insertion and forming sub marginal collective vain. Inflorescence 1-3 in each floral sympodium appearing before or with leaves. Spathe is boat

shaped, ovate; pale pink in colour. Spadix is shorter than spathe which appears with or after leaves. Flowers are unisexual- females are 5-7, on one side of the peduncle towards the lower part and the males are present in the upper part of spadix, stylar region absent, stellate stigma with four lobes. Fruits are berry, 4- 6 angled; ovary is ovoid in shape and single celled with many ovules; parietal placentation. Flowering season starts from May and ends in June.

Ariopsis peltata is seasonal herb which is consumed as food in the form of vegetable (leaves cooked with oil and onion), vadi (rolled leaves along with chana paste) and also fresh leaves are directly added to dal for garnishing purpose<sup>[28]</sup>. The tetrameric nature of lectin isolated from Ariopsis peltata (APLT) is confirmed by electrophoretic separation and haemagglutinin activity. However, the APLT has only two subunits of which tetrameric combination of the five isolectins can be represented as A<sub>4</sub>, A<sub>3</sub> B, A<sub>2</sub> B<sub>2</sub>, AB <sub>3</sub> and B<sub>4</sub> respectively. The APLT consists of subunits A and B are found to be glycosylated and they showed identical sugar specificities <sup>[4]</sup>. In this direction, present investigation is an attempt to recognize the phytochemical components and pharmacological evaluation of Ariopsis peltata for understanding their biological activity required for qualitative standardization of drug samples.

## **Materials and Methods**

## Sample collection

*Ariopsis peltata* were collected from Badal Ghat (on the way of Siddapur to Kumata) region of Uttara Kannada District, Karnataka State. The collected plant was authentically identified using Standard flora such as Flora of Presidency of Bombay, Flora of Presidency of Madras and herbarium specimen were prepared and deposited in Department of Botany, Karnatak University, Dharwad,(Voucher specimen number: SB/GSM/23) for further reference. The collected plant materials were cleaned and shade dried. The dried parts were powdered by using electric grinder, sieved to reduce the size and stored in an air tight container to avoid the effect humidity at room temperature. Further analysis wasdone by using stored samples.

### Macroscopy

Morphological features provides easy way for identification. The organoleptic features like texture, colour, odor and taste were determined for leaf and tuber powder as per standard methods. Histochemical analysis of powder sample were done by using reagents such as iodine, potassium iodide, ferric chloride, concentrated HCl and phloroglucinol with Con. HCl (1:1) <sup>[5, 6, 7]</sup>.

### Microscopy

Anatomical studies were done by using fresh samples. Thin hand sections were taken of fresh leaf, petiole and tuber, stained with safranin (4%) and observed under microscope. Photomicrographs in different magnification were taken by using Axio star plus (Carl zeiss) Bright field/fluorescent modular microscope equipped with Cannon's power shotG2 digital camera.

### **Powder microscopy**

Powder microscopic characters are evaluated by treating powder samples with different chemicals like iodine, potassium iodide, ferric chloride, concentrated HCl and phloroglucinol with Con. HCl (1:1) and were observed under microscope.

#### Physicochemical analysis

Physicochemical parameters including percentage of total ash, water-soluble ash, acid-insoluble ash, sulphated ash, extractive value and nutritive content were investigated by following standard method <sup>[10,23]</sup>. The moisture content was determined bydrying at 105<sup>o</sup>C with the help of loss on drying method <sup>[10]</sup>. Nutritive contents such as ash, moisture, fat, fiber, protein and carbohydrate of leaf and tuber were also determined <sup>[13, 24]</sup>.

#### Fluorescence analysis

Fluorescence analysis of dried powder was carried out using standard procedure. The fluorescence analysis of powdered sample of leaf and tuber were observed under visible light, short UV light and long UV light (at 254nmand 365nm) after treating with various chemical reagents such as sodium hydroxide, hydrochloric acid, ferrous chloride solution, benzene, chloroform, acetone, distilled water etc. <sup>[12, 25]</sup>. The characteristic variation in colour appeared were recorded.

#### Preliminary phytochemical analysis

The powdered material was serially extracted by cold extraction (infusion method) and hot extraction (decoction, soxhlet) method using petroleum ether, chloroform, acetone, ethanol and water <sup>[8]</sup>. These extracts were further subjected for preliminary phytochemical screening using standard procedure to determine the presence of different phytoconstituents <sup>[9]</sup>. Both cold the hot extracts were subsequently concentrated using rotary vacuum evaporator to yield dry residue. The extracts were stored in refrigerator for further use.

#### Thin layer chromatography

The extracts were subjected for TLC analysis by using standard procedure and it is mainly used for the detection of the nature of phytochemical present in plants. Thin layer chromatography was carried out using aluminum plate  $(3 \times 6)$ cm) pre-coated with silica gel. For TLC profiling prepared plant extracts were applied on pre-coated TLC plates by using capillary tubes and developed in a TLC chamber using suitable mobile phase. The developed TLC plates were air dried and observed under ultra violet light at both 254 nm and 365 nm. During this investigation, the different components are separated by the differential migration of solute between two phases; one is stationary phase and another one is mobile phase. Here the main principle of separation is adsorption and adsorbent acts as stationary phase. Depending on the nature of stationary phase, its preparation and different combination of solvents were used for best separation components. The retention factor (R<sub>f</sub>) value is calculated by ratio distance travelled by the solute front on the distance travelled by solvent front.

 $R_F = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvents}}$ 

# Results

## Macrocsopy

Organoleptic analysis reveals that nature of leaf powder is coarse, dark green in color with slightly pungent odour and bitter in taste. Tuber powder is smooth, light creamish in color with characteristic pungento dour with slightly bitter taste. Leaf powder causes irritation to mucous membrane compare to that of tuber powder. Leaf and tuber powder were treated with different reagents to know various cell components present in drug sample are represented in Table 1.

#### Microscopy TS of Leaf

Transverse section of Leaf (Figure 1.) shows single layered, thick walled epidermal cells covered by thin cuticle. The epidermal cells have a rectangular shape with slight convex outer margin. Next to epidermis hypodermis is made up of single layer of palisade cells followed by ground tissues. Ground tissue cells are isodiametric or round with small intercellular spaces with pitted cells and cells filled with starch grains. Crystals are observed in the midrib region. Mesophyll cells are situated near to the lower epidermis with intercellular spaces. The vascular bundles are collateral, closed type scattered within ground tissue; proto-xylem and meta-xylem are placed above the phloem. The xylem faces the adaxial surface of leaf, while the phloem faces abaxial to the epidermis. Phloem consists of companion cells and sieve tubes; xylem consists of vessels, tracheids, fibres and xylem parenchyma.

## **TS of Petiole**

Transvers section of tuber shows single layered, rectangular, thick walled epidermal cells covered by thin cuticle. Below epidermis there is a single layer of hypodermis followed by parenchymatous ground tissues with small intercellular spaces. Starch grains are observed in some cortical cells. Vascular bundles are poly-arch and closed type. Vascular bundles are scattered in the ground tissues where meta- xylem cells are surrounded by proto-xylem and phloem present below the xylem cells. In grooved region the phloem are near to periphery where as the xylem is facing the center.

## TS of Tuber

The transverse section of tuber shows irregular wavy outline. A 4-5 layers of periderm forms the outermost tissue. This is followed by a single layer of epidermis with elongated cells with thick cell wall. The ground tissues are round parenchymatous cells with small intercellular space. Starch grains and crystals are scattered throughout the tissues. The central region comprises elongated vascular strands containing xylem elements. Pith is present in the central region.

### **Powder microscopy**

Powder microscopy analysis of leaf and tuber showed characters such as thick walled epidermal cells, crystals, pitted parenchyma, reticulate and spiral vessels and cells containing simple starch grains (Figure 2). Elongated thick walled fibers with small pits were also observed.

### Physicochemical analysis

Proximate analysis such as ash value, solubility value, nutritive contents and extractive values using different solvents like petroleum ether, chloroform, ethanol and water were calculated and presented in Table 2. Extractive value was high for hot aqueous extract of r leaves  $(7.533 \pm 0.008 \text{ W/w})$  and chloroform extract of tuber  $(1.713 \pm 0.008\% \text{ W/w})$ . Acetone extract showed lowest yield in both hot and cold extracts of leaf  $(0.400 \pm 0.005\% \text{ W/w})$  and  $(0.390 \pm 0.001\% \text{ W/w})$  respectively. Low percentage yield was observed in tuber extract of petroleum ether for both hot  $(0.320 \pm 0.001\% \text{ W/w})$  and cold extract  $(0.286 \pm 0.008\% \text{ W/w})$  respectively. Solubility values was high in aqueous extract for leaf (hot

extract-  $6.700 \pm 0.011\%$  w/w, cold extract -  $5.906 \pm 0.012\%$ w/w) and ethanol extract of tuber (hot extract -1.066  $\pm$ 0.066% w/w, cold extract-  $0.886 \pm 0.008\%$  w/w). The content of ash and moisture was found to be more in leaf than in tuber indicating good storage. The fat  $(0.420 \pm 0.005)$ , fiber (0.326) $\pm$  0.006) and protein (0.486  $\pm$  0.003) values of tuber was high than the leaf sample. Carbohydrate content of tuber was more than that in leaf sample. The nutritive value of leaf (329.76 cal/100g) is lower than that of tuber sample (352.39cal/100g). Physico-chemical studies shown higher ash and moisture content in leaf and these values are nearly equal to earlier observation reported by TERI, of Palghar district, Maharashtra related to the Bio prospecting of the local wild edible plants <sup>[28]</sup>. Subsequently fat, fiber and protein values are more in tuber in the current study is also similar to values reported by earlier workers <sup>[14]</sup>.

### Fluorescence analysis

Fluorescence analysis of leaf and tuber powder was carried out after treating with several chemicals reagents. During present study, fluorescence nature of powder was observed at 254 and 365nm UV light, in visible light and the observations are tabulated in Table 3.

## Phytochemical analysis

Successive extraction was performed by using different solvents with increasing polarity from non-polar to polar. The extract showed sticky nature for all solvents and the colour varied from yellowish browntodark green for tuber; however leaf extracts has dark green colour. Phytochemical screening of different extracts revealed the presence of various bioactive components of which carbohydrates, alkaloids, fats and oils, proteins, amino acid, steroids, glycosides and phenols are the most prominent components of leaf extracts. Among these phytochemicals amino acid, steroids and glycosides are absentin tuber and the result of phytochemical test is presented in Table 4.

### **Chromatographic Study**

Thin layer chromatography for all extracts of Ariopsis peltata obtained by successive extraction methods was carried out to confirm its nature by analyzing TLC chromatograms and to identify active ingredients from the extracts (Fig 3 and 4). In the present study TLC profiling of leaf and tuber extracts was done by using combination of different solvent systems like ethyl acetate: hexane, hexane: acetone and chloroform: acetone. The combination of solvent system in different ratio shows effective results for separation of components. For leaf, TLC analysis of petroleum ether extract revealed the presence of 7compounds having  $R_{\rm f}$ values of 0.24,0.26,0.28,0.34,0.36,0.44,0.48 and 0.24, 0.28, 0.34, 0.36, 0.44, 0.46, 0.48 for hot and cold extracts respectively, when a solvent phase of hexane: acetone (8:2) was used. In another solvent phase hexane with ethyl acetate (6:4), compounds having R<sub>f</sub> of 0.7, 0.72, 0.74,0.76, 0.78, 0.80,0.82 for hot extract and for cold extract it is 0.54, 0.56, 0.62, 0.66, 0.68, 0.70, 0.74, 0.78, 0.80 respectively. Compounds having  $R_{\rm f}$  of 0.82 and 0.80 were most prominent and clear spots were observed for respective extracts. In case of tuber, TLC for aqueous extracts showed the presence of 9 compounds having Rf values of 0.54, 0.56, 0.62, 0.66, 0.68, 0.76, 0.82, 0.86, and 0.90 for hot extract and 8 compounds having R<sub>f</sub> values of 0.54, 0.60, 0.62, 0.66, 0.74, 0.78, 0.82, and 0.86 respectively; when a solvent phase of hexane: acetone (4:6) were used. The  $R_f$  values for both leaf and tuber extract were represented in Table 5 and 6.

Table 1: Table 1. Histochemical Tests of A. peltata
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Descente	Togt for	Colour	Result		
Reagents	Test for	Leaf	Tuber	Leaf	Tuber
Iodine	Starch	Green	Blue	Absent	Present
Ferric chloride solution (10%)	Tannins	Yellow	Yellow	Absent	Absent
Con. Sulphuric acid	Cellulose	Green	Green	Present	Present
Con. HCl + phloroglucinol	Lignin	Greenish Brown	Yellowish brown	Absent	Absent
Con. HCl	Calcium oxalate crystals	No effervescence	No effervescence	Absent	Absent

Table 2: Physicochemical observation of Leaf and Tuber of A. peltata

Parameter	Leaf	Tuber
1. Ash value (% w/w)		
a. Total ash	$0.816 \pm 0.008$	$0.593 \pm 0.008$
b. Acid soluble ash	$0.036 \pm 0.003$	$0.070 \pm 0.005$
c. Water soluble ash	$0.223 \pm 0.003$	$0.200 \pm 0.005$
d. Sulphated ash	$1.476 \pm 0.003$	$0.593 \pm 0.091$
2.Extractive value (% w/w)	Hot extract Cold extract	Hot extract Cold extract
a. Pet. Ether	$2.170 \pm 0.011 \ 2.096 \pm 0.014$	$0.320 \pm 0.001 \ 0.286 \pm 0.008$
b. Chloroform	$0.666 \pm 0.008 \ 0.570 \pm 0.011$	$1.713 \pm 0.008 \ 1.520 \pm 0.010$
c. Acetone	$0.4000 \pm 0.005 \ 0.390 \pm 0.001$	$0.766 \pm 0.006 \ 0.603 \pm 0.008$
d. Ethanol	$5.556 \pm 0.008 \ 5.153 \pm 0.008$	$1.033 \pm 0.008 \ 0.933 \pm 0.008$
e. Water	$7.533 \pm 0.008\ 2.890 \pm 0.011$	$0.403 \pm 0.008 \; 0.403 \pm 0.008$
3. Solubility test	Hot extract Cold extract	Hot extract Cold extract
a. Ethanol	$5.210 \pm 0.010\ 4.986 \pm 0.006$	$1.066 \pm 0.066 \ 0.886 \pm 0.008$
b. Water	$6.700 \pm 0.011 \ 5.906 \pm 0.012$	$0.376 \pm 0.008 \; 0.300 \pm 0.005$
4. Nutritive content (%)		
a. Ash	$0.816 \pm 0.008$	$0.593 \pm 0.008$
b. Moisture	$17.166 \pm 0.033$	$11.833 \pm 0.666$
c. Fat	$0.340 \pm 0.005$	$0.420 \pm 0.005$
d. Fiber	$0.286 \pm 0.003$	$0.326 \pm 0.006$
e. Protein	$0.410 \pm 0.000$	$0.486 \pm 0.003$
f. Carbohydrate	81.26	86.66
5.Nutritive value Cal per 100 g powder	329.76	352.29

## Table 3: Fluorescence analysis of A. peltata

	Visil	ole light	U.V light	at 254nm	U.V light at :	365nm
Tests	Leaf	Tuber	Leaf	Tuber	Leaf	Tuber
P + NaOH (Aqueous)	Light olive green	Linen white	Apple green	Yellow green	Ceylon green	Light green
P + NaOH (Alcoholic)	Apple green	Old lace white	Dark green	Light green	Dark brown	Light green
P + 1N HCl	Light olive green	Old lace white	Olive green	Light green	Ceylon green	Light green
$P + 50\% H_2SO_4$	Light green	Wheat	Parrot green	Brownish green	Light green	Brown
P + 50% HNO <sub>3</sub>	Sandy brown	Olive green	Light green	Yellow green	Light green	Light green
$P + HNO_3$	Tan	Brownish green	Light green	Fluorescent green	Light green	Light green
P + Acetic acid	Olive green	Light brown	Fluorescent green	Yellowish green	Ceylon green	Light green
$P + FeCl_3$	Maroon	Maroon	Black	Black	Black	Black
$P + HNO_3 + NH_3$	Yellowish brown	Sandy brown	Brownish green	Yellow green	Ceylon green	Light green
$P + H_2SO_4$	Light brownish green	Light brown	Dark green	Dark green	Dark brown	Dark brown
$P + NH_3$	Brownish green	Light brown	Light green	Olive green	Light green	Olive
P + Pet. Ether	Light green	Sandy brown	Light green	Apple green	Ceylon green	Olive
P + Methanol	Light algal green	Light brown	Yellowish green	Light green	Light green	Cream
P + Water	Light green	Lavender blush white	Light green	Yellowish green	Brown	Olive
P + Benzene	Light olive green	Light brown	Apple green	Light green	Ceylon light green	Cream
P + Glycerin	Olive green	Blanched almond brown	Apple green	Brownish green	Ceylon light green	Brown
P + Acetone	Light green	Light brown	Yellow green	Light green	Light green	Light green
P + Chloroform	Light algal green	Light brown	Light green	Fluorescent green	Brown	Light green

P: Powder

 Table 4: Phytochemical analysis of A. peltata Leaf and Tuber extracts

Tests			Ether tract		oform ract		tone ract		anol ract		ieous ract
		L	Т	L	Т	L	Т	L	Т	L	Т
Tests for Carbohydrates											
Reducing sugar	Fehling's test	+	+	_	+	_	+	+	+	_	+
	Benedict's test	_	_	_	_	_	_	_	_	_	_
Monosaccharids	Barfoed's test	_	+	_	_	_	_	_	+	_	+
	Bial's test	_	_	_	+	_	+	_	_	_	
	HCl test	_	_	_	_	_	_	_	_	_	_

Non-redusing sugar	Iodine test	_	+	_		+	_	+	_	+	+
Test for Proteins	Biuret test	_	_	_	+	_	_	_	_	_	+
	Millon's test	+	+		_	_	+	_	+	+	_
	Xanthoprotein test	_	_		+	_	+	_	+	+	_
Test for Amino acids	Ninhydrine test	+			_					+	_
	Test for tyrosine									+	
	Test for tryptophan					+				+	
	Test for Cysteine	+	_						_	+	
Fats and Oils	Sudon red iii test	+	+			+	+		_	_	+
	Filter paper test		_			+	+		_		_
	Solubility test	+	+		+				_		
	Saponification test	+	+		+	_			_		
Steroid test	Salkowski test					_				+	
	Liebermann- burchard reaction	+	_	+		+		+	_		
	Liebermann reaction	+		+		+		+			
Volatile oils											
Test for Glycosoides									_		
Test for cardiac glycosoides	Liebermann's test	+		+		+					
Test for saponine glycosoides	Alkalinity test	+				+			_		
	Fluorescence test	+	_			+			_		
Test for cyanogenetic	Shinoda test										
glycosoides		-	-	-	-	-	-	-	-	-	-
	Nao H test	_	_		_	_	_	_	_		
Test for Alkaloides											
	Draganoff's test	+	+	_	+	_	_	_	_	_	_
	Mayer's test	+	+	I	+	_	_	_	_	_	_
	Wagner's test	+	+	+	+	_	_	_	_	_	_
Test for Tannins and Phenolic compounds	5%FeCl <sub>3</sub> solution	_	+	+	+	_	-	-	+	_	-
	Acetic acid solution	_	_	_	+	_	+	_	_	+	_
	Potassium dichromate	_	_	+	_	_	_	_	+	+	_
	NH4OH + AgNO3				+	+	+	+	+	_	
	Dil.HNO <sub>3</sub>		+	+	_	_			_		
Test for Enzymes											
Catalase	H <sub>2</sub> O <sub>2</sub> test				_	_				_	_
Dehydrogenase test	TTC test				+	+					
	Methylene blue test										
Test for Organic acids	Oxalic acid				t –	1 -				+	<u> </u>
	Malic acid		-		<u> </u>					1	<u> </u>
	Citric acid		-		-					+	
Test for Vitamin			-		-						
Vitamin D	Ferrous sulphate test			+							
· Leaf T: Tuber	r enous surplute test	_	L —	1		I —	I —	L —	I —	L —	L —

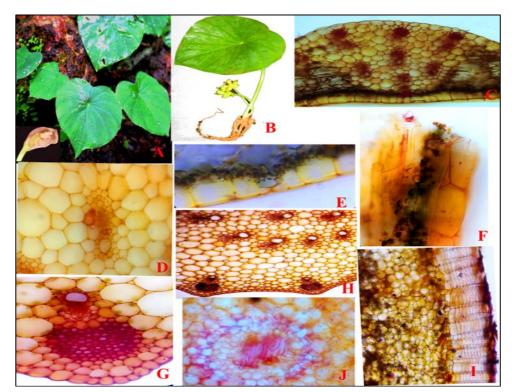
L: Leaf, T: Tuber

# Table 5: TLC of A. peltata Leaf extracts

Extract / solvent combination	<b>R</b> <sub>f</sub> value				
Extract / solvent combination	Hot extract	Cold extract			
Pet ether extract Hexane: Acetone (8:2)	0.24,0.26,0.28,0.34,0.36,0.44,0.48	0.24, 0.28, 0.34, 0.36, 0.44, 0.46, 0.48			
Chloroform extract Hexane: Ethyl acetate (6:4)	0.7, 0.72, 0.74, 0.76, 0.78, 0.80, 082	0.54, 0.56, 0.62, 0.66, 0.68, 0.70, 0.74, 0.78, 0.80			
Acetone extract Hexane :Ethyl acetate (8:2)	0.64, 0.66, 0.70, 0.72, 0.74, 0.78	0.64, 0.66, 0.70, 0.74, 0.76, 0.78			
Ethanol extract Hexane :Ethyl acetate (8:2)	0	0.44,0.54, 0.58			
Aqueous extract Hexane : Acetone (8:2)	0.24, 0.28, 0.36, 0.42	0.08, 0.14, 0.16, 0.18, 0.24, 0.28, 0.32			

# Table 6: TLC of A. peltata Tuber extracts

Enter of / solution to on the sting	Rfvalue					
Extract / solvent combination	Hot extract	Cold extract				
Pet ether extract Hexane: Acetone (8:2)	0.76, 0.80	0.38, 0.44				
Chloroform extract Hexane: Ethyl acetate (6:4)	0.50, 0.54, 0.56, 0.62	0.48, 0.50, 0.54, 0.62				
Acetone extract Ethyl acetate: Acetone (6:4)	0.84, 0.88, 0.90	0				
Ethanol extract Chloroform: Acetone(6:4)	0.88, 0.90, 0.92	0				
Aqueous extract Hexane : Acetone (4:6)	0.54, 0.56, 0.62, 0.66, 0.68, 0.76, 0.82, 0.86, 0.90	0.54, 0.60, 0.62, 0.66, 0.74, 0.78, 0.82, 0.86				



**Fig 1:** (A-J) Microscopic analysis of *A. peltata* (1A) Habit (1B) Plant with Fruit (1C) T.S of leaf (1D) xylem and phloem (1E) Structure of stomata (1F) T.S of leaf showing xylem element (1G) T. S. of petiole showing xylem and phloem element with starch grains (1H) T.S of petiole (1I) T.S of tuber with outer periderm (1J) Vascular strand with xylem element

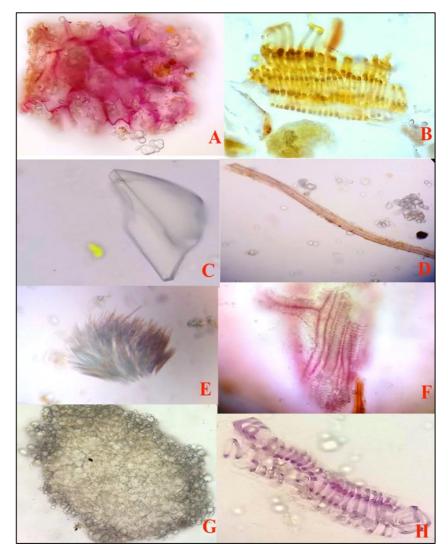


Fig 2: (A-D) Powder and Maceration analysis of *A. peltata* (2A) epidermal cells (2B) reticulate vessels (2C) Cluster shaped crystal (2D) Fiber libri form (2E) Needle shaped crystal (2F) Spiral vessels (2G) Starch grains (2H) Reticulate vessels

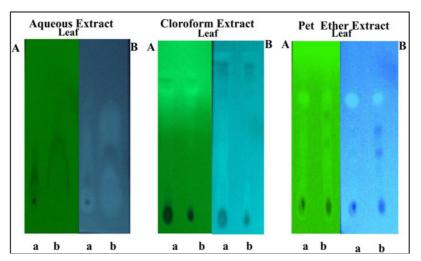


Fig 3: TLC profile for A. *peltata* Leaf extracts TLC analysis of Leaf: Aqueous, Chloroform, Pet ether extracts. A -UV at 254nm, B -UV at 365nm. a- cold extract, b- hot extract.

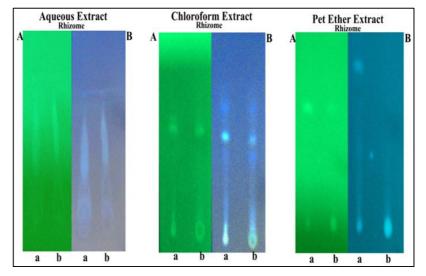


Fig 4: TLC profile for *A. peltata* Tuber extracts TLC analysis of Tuber: Aqueous, Chloroform, Pet ether extracts. A- UV at 254nm, B- UV at 365nm. a- cold extract, b- hot extract.

## Discussion

The pharmacological as well as physicochemical parameters play vital role in identification and standardization of crude drugs. The phytoconstituents which are recorded from present analysis are carbohydrates, alkaloids, fats and oils, proteins, amino acid, steroids and glycosides and phenolic compounds. The presence of these active metabolites could make Ariopsis peltata useful for treating different human ailments and thus providing a potential drug for human use. During present investigation cold (infusion method) and hot (decoction, soxhlet) extraction method were used. Of these, cold extraction is suitable for thermolabile compounds, where hot extraction method is best for thermos table compounds <sup>[18]</sup>. Ethanol and aqueous are most preferred solvents for dissolution of majority of compounds. It is confirmed from the present investigation that the activity of extract affected by method of extraction. However, there is no significant difference in extractive values of both hot and cold extracts.

Along with phytochemical evaluation, macroscopic and microscopic characters are also important tools for identification and quality assessment of herbal drug. Some distinctive features of pitted parenchyma, reticulate and spiral vessels, crystals and starch grains are observed. In the present study, the extractive value and solubility value found useful to evaluate specific chemical constituent and dry yield in different solvents. Ash value analysis plays an important role in determination of purity of drug indicating the presence or absence of inorganic matter such as soil or silica. A high ash value in leaf is an indication of existence of extraneous matter in the crude drug. Moisture content is determined by loss on dry method, is essential for evaluation of stability of crude drug <sup>[24]</sup>.

Fluorescence analysis showed that some chemical constituents present in plant extract showed characteristic fluorescence when illuminated with suitable reagents. The compounds that are not naturally fluorescent to themselves are treated with different reagent to achieve fluorescence <sup>[15,</sup> <sup>26]</sup>. Thin layer chromatography is a very effective technique for separation, identification and estimation of different bioactive components of an extract. The pattern of bands developed on TLC plates provides primary data to evaluate components in plant extracts. In present study TLC analysis of cold extract showed more number of compounds in leaf extract than in hot extract but tuber resulted in more number of compounds in hot extract. Preliminary standardization done for Ariopsis peltata is very useful in future pharmacological studies.

#### Conclusion

Plants are recognized in the pharmaceutical industry for their wide range of pharmacological activities. Phytochemicals are active components of plants and are used as a direct source of crude drug. *Ariopsis peltata* is a good source of vegetable with high nutritional value and closely related species are known to be medicinal. Therefore phytochemical screening of the extracts and pharmacological evaluation are necessary to check the efficacy of *Ariopsis peltata*. The present study clearly depicts that *Ariopsis peltata* can be used as a potential source for a synthesis of new plant based drugs.

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