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Standardization of alkaloid containing drugs

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ABSTRACT

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties. Also some synthetic compounds of similar structure are attributed to alkaloids. In this research, we study the alkaloid containing drugs with Immunomodulator activity. The present study was carried out to investigate morphological, microscopical, physicochemical and phytochemical screening of *Piper longum* (fruit), *Tinospora cordifolia* (stem) and *Aconitum heterophyllum* (root). Morphological studies showed the presence of various diagnostic characters. In microscopical study, T.S of *Piper longum* fruit shows 6 to 12 fruitlet, stem of *Tinospora cordifolia* shows uppermost layer of cork, differentiating into outer zone of thick-walled brownish and compressed cells and root of *Aconitum heterophyllum* shows single layered epidermis consisting of light brown tabular cells rupturing on formation of cork. Ash value, extractive value, foreign organic matter,moisture content and TLC were determined for quality standard of drugs. The result of the study could be useful for identification and preparation of monograph of the plant.

KEYWORDS

Aconitum heterophyllum;
Physicochemical parameter;
Phytochemical screening;
Piper nigrum;
Standardization;
Tinospora cordifolia.

INTRODUCTION

The name "alkaloids" (German: *Alkaloide*) was introduced in 1819 by the German chemist Carl F.W. Meissner, and is derived from late Latin root *Latin:* alkali (which, in turn, comes from the Arabic *al-qalwī* - "ashes of plants") and the suffix *Greek: -οειδής* - "like". However, the term came into wide use only after the publication of a review article by O. Jacobsen in the chemical dictionary of Albert Ladenburg in the 1880s[1].

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. This group also includes some related compounds with neutral[2] and even weakly acidic properties[3]. Also some synthetic compounds of similar structure are attributed to alkaloids[4]. There are many alkaloid containing herbs with good therapeutic efficacy available in India. In this research, we study the alkaloid containing drug with Immunomodulator activity. There are various herbs with immunomodulator activity available in market but commonly drugs are- *Piper longum, Tinospora cordifolia, Aconitum heterophyllum*[5].

Piper longum Linn.(Piperaceae)

Commonly named as Pippali. It is considered as a native of south Asia and is found both wild as well as cultivated, throughout the hotter part of India from cen-

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tral to the north-eastern Himalayas; the herb also grows wild in Malaysia, Singapore, Bhutan[6]. Piper longum contains Essential oil and Alkaloids[7]. Piper longum is used as bioavailability enhancer, digestive, in treatment of bronchitis and also hepatoprotective agents[8]. The Piper species contain the piperidine type of alkaloid, piperine which is a central nervous depressant. Piperine the alkaloid is responsible for the pungency of *P.nigrum* L. and *P. longum* L.[9]. *Piper longum* possess anti-platelet effect[10], anti-asthmatic effect, antidepressant activity, antithyroid activity, antioxidant, fertility Enhancer, antitumor activity. It has also antiamoebic activity and stimulant effect[11], analgesic activity, immunomodulatory[12].

Tinospora cordifolia (Adoraceae)

Commonly named as Guduchi is a large, glabrous, deciduous climbing shrub. It is distributed throughout tropical Indian subcontinent and China. In Hindi, the plant is commonly known as Giloya, which is a hindu mythological term thatrefers to the heavenly elixir that have saved celestial beings from old age and kept them eternally young[13,14]. The main consistuents of Tinospora cordifolia is Terpenoids and Alkaloids. *Tinospora cordifolia* possess antileprotic, antistress, anti-malarial activities[15], anticancer, antidiabetic and hyperglycaemic activity, antiinflamatory, hypolipidaemic, antioxidant, antistress, antiulcer, immunobiological activity[16], liver disorder, mental disorder, hepatic disorder, stomachic, diuretic[17].

Aconitum heterophyllum L.

(Ranunculaceae). It is commonly called as Shudha Vatsnabh. It is found in the alpine Himalayas from Sikkim to Garhwal & Assam. It mainly contain alkaloids. Root posses depressant activity, but after mitigation in cow's milk for 2-3 days, they exhibit stimulant activity[18]. It is used as Narcotic, Sedative, antimicrobial, antiinflammatory. It posses antiperiodic, analgesic, antitussive, antidiarrhoeal, dyspepsia, antipoisonous activity[19].

The objective of the present study was to establish various Pharmacognostic standards and to evaluate preliminary phytochemical and physicochemical analysis that can facilitate identification and assist in the preparation of monograph of the plant.

MATERIALS AND METHODS

Plant material

The dried fruit of *Piper longum*,stem of *Tinospora cordifolia*, and root of *Aconitum heterophyllum* were collected from local market of Jaipur in the month of October. The shade dried powder was used for the determination of macroscopic, microscopic, physicochemical parameters and phytochemical screening.

Macroscopical studies

The dried fruit, stem, root of *Piper longum, Tinospora cordifolia, Aconitum heterophyllum* respectively were subjected to macroscopical studies which comprised of organoleptic characteristics of the drug viz., size, colour, odour, taste, shape.

Microscopical studies

Qualitative microscopic evaluation was carried out by taking transverse sections of fruit, stem and root of piper, tinospora and aconitum. Free hand section of softened fruit, stem and root were boiled with chloral hydrate to remove all the coloring matter and then carefully stained with phloroglucinol and hydrochloric acid. The sections were transferred to mounted (glycerin) on a slide and a cover slip was placed over it. Powder characteristics of fruit, stem and root powder were also studied using reported method[20].

Physicochemical parameters

Various physicochemical parameters such as total ash, water soluble ash, acid insoluble ash, sulphated ash, water extractable matter, alcohol extractable matter, foreign matter, moisture content, were calculated[20,21].

Preliminary phytochemical screening

The aqueous and alcoholic extracts were subjected to qualitative chemical examination for the identification of various plant constituents. Following tests were performed.

Tests for carbohydrates and glycosides

200 mg of aqueous extract was dissolved in 5ml of distilled water and this solution was subjected to Molisch test for the detection of carbohydrates. Small portion of the extract was hydrolyzed with dilute hydrochloric

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acid for few hours in water bath and was subjected to Liberman-Burchard's test, Legal's and Borntager's test to detect the presence of different glycosides. Another small portion of extract was treated with Fehling's reagent, Barfoed reagent to detect the presence of various sugars. For the detection of saponin glycosides, Foam test and Hemolytic tests were carried out[20,21].

Tests for protein and free amino acids

A small quantity of alcoholic extract was dissolved in few ml of water and was subjected to Millon's test, Biuret test and Ninhydrine test[20,21].

Tests for phenolic compounds and tannins

Small quantities of alcoholic extracts were treated with 5 % $FeCl_3$ solution, 1 % of gelatin containing 10 % NaCl, 10 % lead acetate and aqueous bromine solution for the detection of phenolic compounds and tannins[20,21].

Tests for alkaloids

The small portion of dried alcoholic extract was stirred with a few drops of dilute Hydrochloric acid and was filtered. The filtrate was tested with various alkaloidal reagents such as Mayer's reagent, Dragendroffs reagent, Hager's reagent, Wagner's reagent[20,21].

RESULTS

Macroscopical studies

The macroscopic character was useful in quick identification of plant material and also serves as an important standardization parameter (TABLE 1).

TABLE 1 : Macroscopical study

Sr. No	Biological Name	Plant part	Image	Organoleptic character
1.	*Piper longum Linn.* Family- Piperaceae	Fruit		**Shape**- Cylindrical **Colour**- Greenish black to black **Odour**- Aromatic **Taste**- Pungent **Size**- 2.5-3.0 cm length
2.	*Tinospora cordifolia* Miers. Family- Menispermaceae	Stem		**Shape**- Cylindrical **Colour**- Brownish **Odour**- Astringent **Taste**- Bitter **Size**- 1-4 cm length 0.25-0.5 cm in diameter
3.	*Aconitum heterophyllum* Linn. Family- Ranunculaceae	Root		**Shape**-Longitudinal and tapering at one end **Colour**- Creamish brown **Odour**- Indistinct **Taste**- Bitter **Size**- 5-6 cm length 1-2 cm in diameter

Microscopic study

Piper longum

Transverse Section (T.S.) of fruit shows 6 to 12 fruitlet, arranged in circle on a central axis, each having an outer epidermal layer of irregular cells filled with deep brown content and covered externally with a thick cuticle; mesocarp consists of larger cells, usually collapsed, irregular in shape and thin-walled; endocarp

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and seed coat fused to form a deep zone, outer layer of this zone composed of thin-walled cells and colourless, inner layer composed of tangentially elongated cells, having reddish-brown content; most of endocarp filled with starch (figure 1(i)).

Powder Microscopy of *Piper nigrum* fruit shows:Fragments of parenchyma, Oil globules, round to oval starch grains, Fibres, oval to elongated stone cells (figure 1(ii)).

Tinospora cordifolia

Transverse section of stem shows outer-most layer of cork, differentiating into outer zone of thick-walled brownish and compressed cells, inner zone of thin

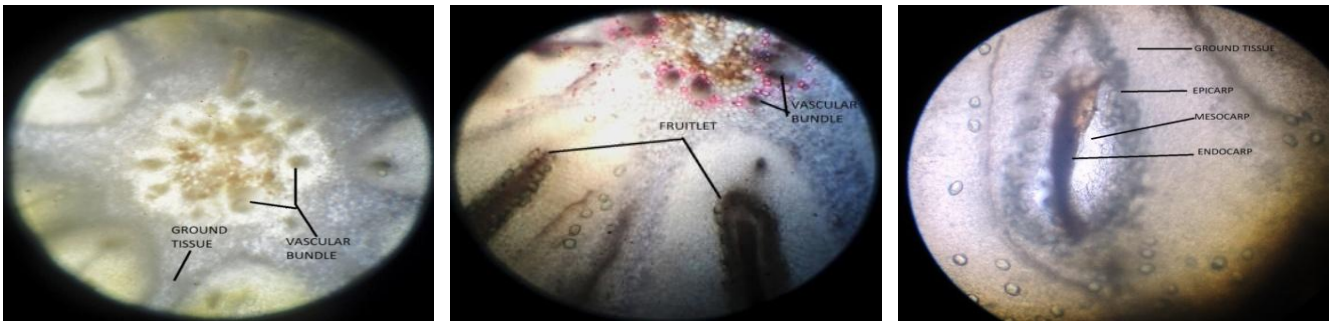
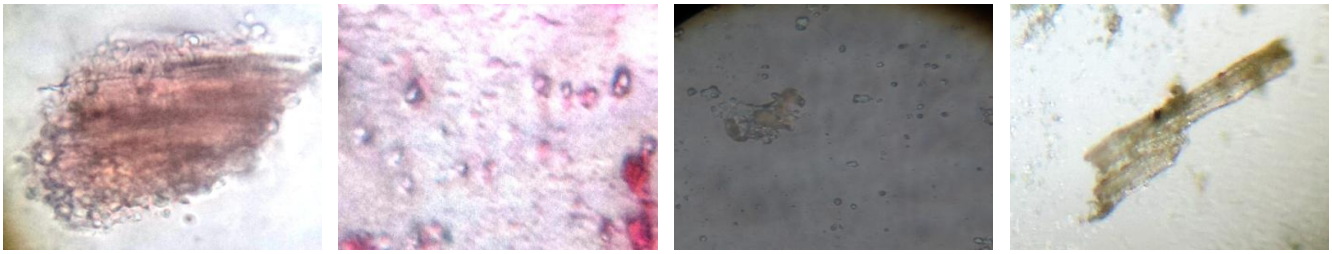


Figure 1(i) : Plant anatomy of *Piper longum*



Parenchyma **Oil globule** **Starch grain** **Fibre**

Figure 1(ii) : Powder microscopy of *Piper longum*

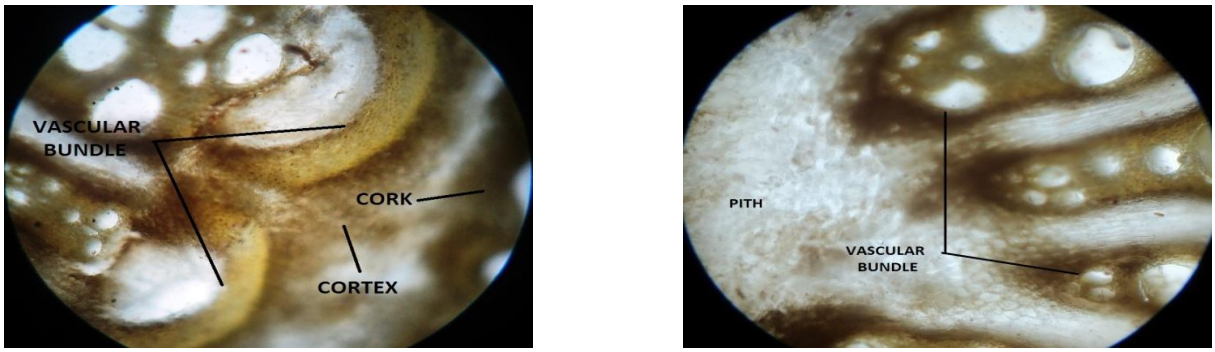
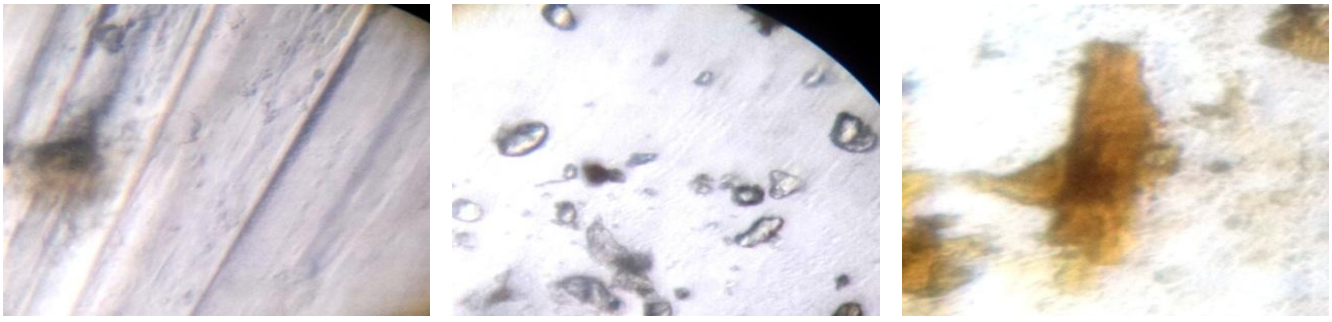


Figure 2(i) : Plant anatomy of *Tinospora cordifolia*



Fibre **Strach grain** **Vessel**

Figure 2(ii) : Powder microscopy of *Tinospora cordifolia*

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walled colourless, tangentially arranged 3-4 rows of cells, cork broken at some places due to opening of lenticels, followed by 5 or more rows of secondary cortex of which the cells of outer rows smaller than the inner one, vascular zone composed of 10-12 or more wedge-shaped strips of xylem, externally surrounded by semi-circular strips of phloem, alternating, with wide medullary rays (figure 2(i)).

Powder Microscopy of *Tinospora cordifolia* stem shows Fibre, Starch grain, Vessel (figure 2(ii)).

Aconitum heterophyllum

Transverse section of root shows, single layered epidermis consisting of light brown tabular cells rupturing on formation of cork,cortex much wider consisting of tangentially elongated or rounded, thin walled parenchymatous cells with intracellular spaces with starch grains (figure 3(i)).

Powder Microscopy of Aconitum heterophyllum root shows Vessel, Starch grain, Fibre (figure 3(ii)).

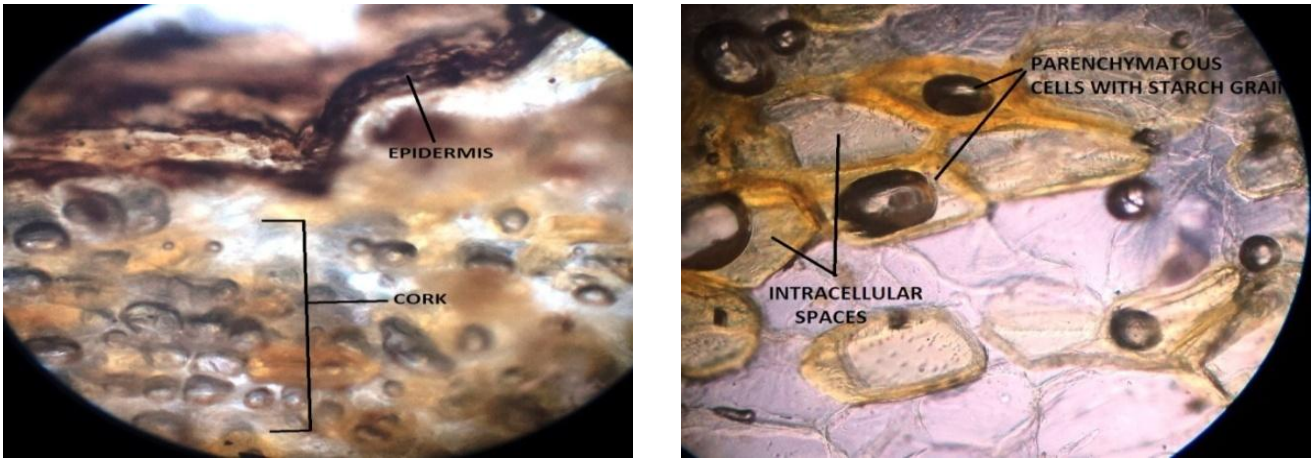
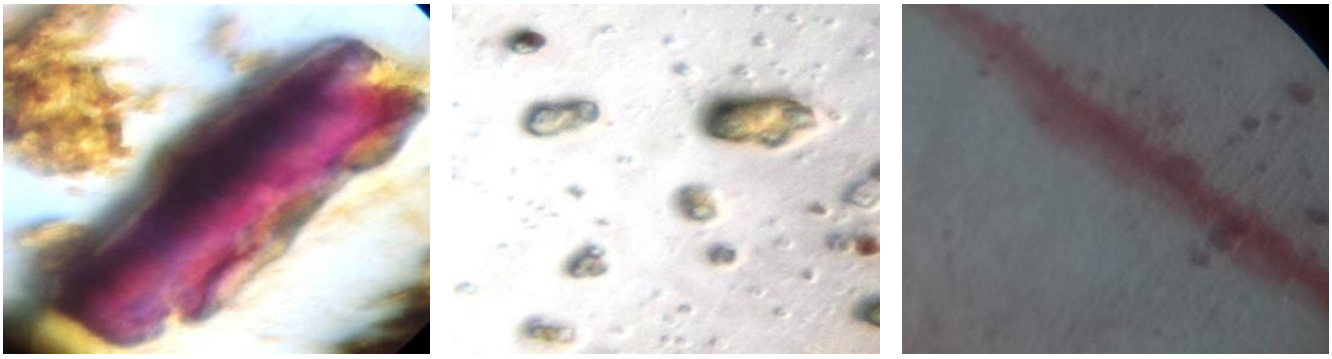


Figure 3(i) : Plant anatomy of *Aconitum heterophyllum*



Starch grain **Fibre** **Vessel**

Figure 3(ii) : Powder microscopy of *Aconitum heterophyllum*

Physicochemical parameter

The ash value, extractive value, moisture content, foreign matter of all drugs were shown in (TABLE 2).

Phytochemical screening

The phytochemical test for aqueous and methanolic extracts were shown in (TABLE 3).

TABLE 2(i) : Foreign matter, loss on drying

SNO	CONSTITUENTS	FOREIGN MATTER		LOSS ON DRYING	
		INFERENCE (API*)	OBSERVED	INFERENCE (API*)	OBSERVED
1.	*Piper longum*	NMT 2%	0.8%	-------	2.62%
2.	Tinospora cordifolia	NMT 2%	0.8%	------	5.2%
3.	*Aconitum heterophyllum*	NMT 2%	0.1%	------	4.0%

API – Ayurvedic Pharmacopoeia of India, NMT – Not More Than

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TABLE 2(ii) : Ash value

SNO	CONSTITUENTS	TOTAL ASH		ACID INSOLUBLE ASH		WATER SOLUBLE ASH	SULPHATED ASH
		INFERENCE (API*)	OBSERVED	INFERENCE (API*)	OBSERVED		
1.	*Piper longum*	NMT 7%	4.5%	NMT 0.5%	0.3%	4.0%	2.33%
2.	*Tinospora cordifolia*	NMT 16%	7.2%	NMT 3%	1.5%	3.8%	5.23%
3.	*Aconitum heterophyllum*	NMT 5.5%	3.1%	NMT 2%	1.2%	1.8%	2.18%

API – Ayurvedic Pharmacopoeia of India, NMT – Not More Than

TABLE 2(iii) : Extractive value

S.No	Consistuents	Water soluble		Alcohol soluble		Chlorofom soluble	Pet. ether soluble	Benzene soluble	Ethyl acetate soluble	Acetic acid soluble
		STD (API*)	OBS	STD (API*)	OBS					
1.	*Piper longum*	NLT 7%	10%	NLT 5%	7.12%	0.5%	1.7%	1.23%	0.8%	0.63%
2.	*Tinospora cordifolia*	NLT11%	15.7%	NLT 3%	6.19%	0.89%	1.73%	3.12%	0.21%	0.5%
3.	*Aconitum Heterophyllum*	NLT24%	25.1%	NLT 8%	10.2%	1.2%	5.19%	3.5%	2.1%	0.89%

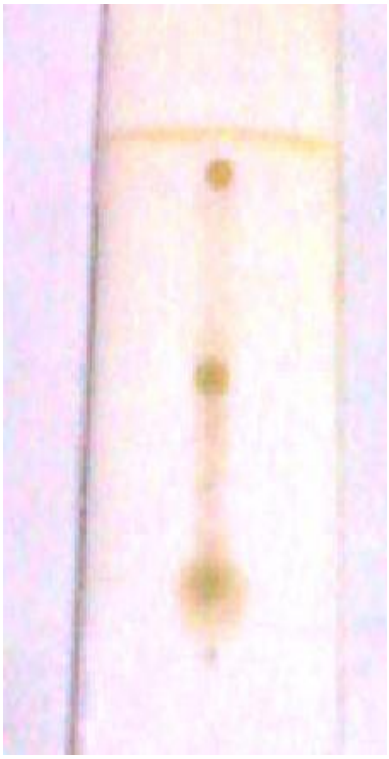
API – Ayurvedic Pharmacopoeia of India, NLT – Not Less Than

Qualitative TLC analysis of *Piper longum*

Sample Std

Sample-
Methanolic extract
Observed Rf of sample- 0.34, 0.47, 0.5
Standard- Piperine
Observaed Rf of standard- 0.49
Solvent system-
Toluene : Ethyl acetate
(8 : 2)
Visualizing agent- Iodine vapour

Qualitative TLC analysis of *Tinospora cordifolia*



Sample

Sample-
Methanolic extract
Observed Rf of sample-
0.1,0.5,0.9
Solvent system-
Benzene : pet. ether
(3 : 1)
Visualizing agent-
Iodine vapour

Qualitative TLC analysis of *Aconitum heterophyllum*

Sample Sample

Sample-
Methanolic extract
Observed Rf of sample-
0.1,0.78,0.86
Solvent system-
Chloroform : Methanol
(9 : 1)
Visualizing agent-
Iodine vapour

Figure 4 : Thin layer chromatography

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TABLE 3 : Phytochemical determination

SNO	CONSTITUENT		CRB	ALK	PRT	GLY	TAN	FLV	STR	VO
1.	*Piper longum*	Aq Ext	+	+	-	-	-	-	-	+
		Alc Ext	+	+	-	-	-	+	-	+
2.	Tinospora cordifolia	Aq Ext	+	+	-	+	+	+	+	-
		Alc Ext	+	+	-	+	+	+	+	-
3.	*Aconitum heterophyllum*	Aq Ext	-	+	-	+	-	+	-	-
		Alc Ext	-	+	-	+	-	+	-	-

CRB – Carbohydrate, ALK- Alkaloid, PRT- Protein, GLY- Glycoside, TAN- Tannin, FLV- Flavonoid, STR- Steroid, VO- Volatile Oil, Aq- Aqueous, Alc- Alcoholic., (-)= Absent, (+) = Present.

Thin layer chromatography

TLC of methanolic extract of all drugs were shown in (figure 4).

CONCLUSION

In present investigation various standardization parameters such as macroscopical, microscopical, physicochemical parameters and phytochemical screening of *Piper longum, Tinospora cordifolia and Aconitum heterophyllum* was carried out. In this scenario, anatomical studies have become mandatory for proper identification. Thus, our study is an important landmark in correct identification of PLANTS.

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