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Pharmacognostical and Preliminary Phytochemical Screening of *Nardostachys jatamansi Dc* Rhizome, *Achyranthus aspera Linn* plant and *Trachyspermum ammi Linn* fruit

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ABSTRACT

Nardostachys jatamansi, Achyranthus aspera and Trachyspermum ammi are very important medicinal plants which are traditionally used in many medical conditions. The present study involves Pharmacognosy and preliminary phytochemical investigations of the rhizomes, whole plant and fruit parts of above three plants respectively .This study consist of the morphological and microscopical study of the plant; the phytochemical screening and testing for alkaloids, glycosides, tannins, steroids and flavonoids; TLC study and HPTLC fingerprinting. The parameters from the above were recorded with an objective of drawing an attention on the plant as well as a reference for further scientific investigations.

Keywords: *Nardostachys jatamansi, Achyranthus aspera, Trachyspermum ammi,* Flavanoids, Microscopical, Pharmacognosy.

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1. INTRODUCTION

1.1 ***Nardostachys jatamansi DC*** is a small, perennial, dwarf, hairy, rhizomatous, herbaceous, endangered and most primitive species within family *Valerianacae.* Many terpenic and coumarin derivatives were isolated from rhizomes.[1] Jatamansi has been widely used for medicine and in perfumery for centuries in India. It is valued for anti-lipid peroxidative, hypolipidemic, antioxidant, hepatoprotective, sedative, tranquilizing, antihypertensive, anti- inflammatory, antidepressant-like activity, anticonvulsant activity and hypotensive properties, anti-asthmatic and anti-estrogenic activity.[2]

1.2 ***Achyranthus aspera Linn*** (Latjeera) is an erect or procumbent, annual or perennial herb of about 1- 2 meter in height[3] Traditionally, the plant is used in asthma and cough. It is pungent, antiphlegmatic, antiperiodic, diuretic, purgative and laxative, useful in oedema, dropsy and piles, boils and eruptions of skin etc. The content of free oleanolic acid in *A. aspera* roots is 0.54 % [4,5]. From the roots ecdysterone and oleanolic acid have been isolated. In the unripe seeds saponines, oleanolic acid, amino acids and hentriacontane, a long chained carbohydrate, have been found. In the shoots an aliphatic dihydroxyketone 36, 37-dihydroxyhenpentacontan-4-on and triacontanol could be found. [6]

1.3 ***Trachyspermum ammi Linn*** Sprague syn. *Carum copticum Benth. and Hook (Fam. Umbelliferae)* commonly known as ajwain is an annual, erect herb, aromatic, with striate stem, white flowers and small brownish fruit up to 90 cm tall. Ajwain seed analysis has revealed it to contain fibre (11.9%), carbohydrates (38.6%),tannins, glycosides, moisture (8.9%), protein (15.4%), fat (18.1%), saponins, flavone and mineral matter (7.1%) containing calcium, phosphorous, iron and nicotinic acid.[7] The plant is used traditionally as a stimulant, carminative, flatulence, atonic dyspepsia, diarrhoea, abdominal tumours, abdominal pains, piles, and bronchial problems, lack of appetite, galactogogue, asthma and amenorrhoea.

1.4 The present study was undertaken for Pharmacognostic and Phytochemical study of extract of three plant and study of TLC with HPTLC fingerprinting for better separation of chemical compounds from individual plants..

2. MATERIALS AND METHODS

2.1 Collection and Authentication of plant:

Rhizome of *Nardostachys jatamansi DC,* Whole plant of *Achyranthus aspera Linn* and fruit of *Trachyspermum ammi Linn* were collected from Local market of Rajkot. The plants were identified and authenticated at Department of Botany, Saurashtra University, Rajkot. Further, the plants were identified by comparing it morphologically and microscopically with the description given in different standard texts and floras .[8-13] Fresh plant materials were cleaned dried at room temperature and powdered.

2.2 Macroscopic Observation: [8-13]

Rhizome of *Nardostachys jatamansi DC,* Whole plant of *Achyranthus aspera Linn* and fruit of *Trachyspermum ammi Linn* were subjected to macroscopic studies which comprised of organoleptic characters of the viz., color, odor, appearance, taste, smell, texture, fracture, etc. and compared with standard references.

2.3 Photomicrography of anatomical study of plant materials:

Stained and unstained transverse section of all the 3 plants material were checked and compare with standard references and the photomicrography of the sections at different magnifications were also recorded using Olympus CH20i microscope attached with Magnus MIPS camera.

2.4 Physicochemical constants:[12-16]

Physicochemical parameters like

Foreign organic matter; Determination of moisture content; Ash values e.g.(a) Total ash (b) Acid insoluble ash (c) Water soluble ash; Extractive values e.g.(a) Hot percolation (b) Cold maceration such as Ethanol soluble extractive, Water-soluble extractive, Ether soluble extractive; Foaming index; Determination of swelling index were checked for all three plants individually.

2.5 Phytochemical study:

2.5.1 Preparation of Extract: [10, 12]

The Powdered plant material was repeatedly extracted in a Soxhlet apparatus using different solvents according to increase in polarity, starting from Petroleum ether followed by Benzene, Chloroform, Ethyl acetate, Methanol, Aqueous (Chloroform: water-1:99).The extracts were evaporated and concentrated. The concentrate extracts were used for phytochemical analysis of different extracts for individual plants.

2.5.2 Phytochemical analysis of different extracts of Rhizome of *Nardostachys jatamansi DC,* Whole plant of *Achyranthus aspera Linn* and fruit of *Trachyspermum ammi Linn:*[14,18]

The extract obtained from successive solvent extraction were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids, glycosides, carbohydrates, phenolics and tannins, phytosterols, fixed oils and fats, proteins and amino acids, flavonoids, saponins, gums and mucilage using reported methods.

2.6 Identification by TLC (Thin Layer Chromatography):[13,19]

2.6.1 TLC profile of Hydroalcoholic (50%) Extract of *Nardostachys jatamansi DC* rhizome:

- **Chromatographic conditions**
- **Solvent system:** Petroleum ether: acetone (3:1)
- **Test solution:** dried Hydro alcoholic extract in sufficient ethanol.
- **TLC estimation**: The test samples were applied on TLC plate prepared with silica gel-G (activated) having a thickness of about 0.5mm.The chromatogram was developed in Petroleum ether: acetone (3:1). The plate was air dried and sprayed with spraying reagent.
- **Detection:** under the UV range of 254nm. Plate was also observed after derivatization with anisaldehyde sulphuric acid and heated at 105°C for 10- 15 min.

2.6.2 TLC profile of Hydroalcoholic (50%) Extract of *Achyranthus aspera Linn* whole plant:

- **Chromatographic conditions**
- **Solvent system:** Toluene: ethyl acetate: formic acid (4.5: 0.5: 0.1)
- **Test solution:** dried Hydro alcoholic extract in sufficient ethanol.
- **TLC estimation**: The test samples were applied on TLC plate prepared with silica gel-G (activated) having a thickness of about 0.5mm.The chromatogram was developed in Toluene: ethyl acetate: formic acid (4.5: 0.5: 0.1). The plate was air dried and sprayed with spraying reagent.
- **Detection:** After spraying with 10 % H_2SO_4 in ethanol and dried at room temperature.

2.6.3 TLC profile of Hydroalcoholic (50%) Extract of *Trachyspermum ammi Linn* fruit:

- **Chromatographic conditions**
- **Solvent system:** Toluene: Ethyl acetate (9.3: 0.7)
- **Test solution:** dried Hydroalcoholic extract in sufficient ethanol.
- **TLC estimation**: The test samples were applied on TLC plate prepared with silica gel-G (activated) having a thickness of about 0.5mm.The chromatogram was developed in Toluene: Ethyl acetate (9.3: 0.7). The plate was air dried and sprayed with spraying reagent.
- **Detection:** sprayed with anisaldehyde sulphuric acid reagent followed by heating at 110°C for 5-10 min.

2.7 HPTLC finger print profile for hydroalcoholic (50%) extract of all three plants:[13,19]

Stationary Phase: 10×10 cm Aluminum Precoated Silica gel 60 GF_{254} Plate (0.2 mm thickness) of Merck Pvt. Ltd. (India)

Solvent system: same as in TLC for all three plants

Test solution: Dissolve 100 mg dried Hydro alcoholic extract in 10 ml ethanol.

HPTLC estimation: The test samples were applied in volumes of 4, 6, 8, 10, 11, 12 and 13 ml on TLC aluminum plates pre coated with silica gel. The chromatogram was developed in solvent system as per in TLC. The plate was air dried and scanned at 366 nm in absorbance mode. The air-dried Achyranthus aspera HPTLC plates were directly viewed after derivatization with 10 % H_2SO_4 in ethanol.

3. RESULT AND DISCUSSION

3.1 Morphology of plant: Fig.3.1.1, 3.1.2 & 3.1.3 shows the morphology of plant.

Fig.1 ***Nardostachys jatamansi rhizo*****me**

3.1.1 Macroscopic observation of *Nardostachys jatamansi DC* rhizome.

Botanical Name: *Nardostachys jatamansi DC or Nardostachys grandiflora* DC

English Name: Musk-root, Indian spikenard, Indian nard

Family: Valerianaceae

Nardostachys jatamansi DC (Family Valerianaceae), commonly known as bhutjata, nalada or spikenard, is an erect perennial herb, 10-60m high with long, stout, woody rootstock.

Dried rhizomes are 2.5 to 7.5 cm in length, cylindrical, covered by a bundle of fine reddish brown fibres. Outer layer dark grey or brown, inner layer brown or yellow. Fracture brittle. Odour highly agreeable, aromatic; taste bitter and pungent.

Fig.2 ***Achyranthus aspera*** **plant**

3.1.2 Macroscopic observation of *Achyranthus aspera Linn* whole plant.

Botanical Name: *Achyranthus aspera Linn*.

Common Name: Apaamaarga, Safad Aghedo, Shikhari, Latjira.

Family: Amaranthaceae

Achyranthus aspera Linn (Latjeera) is an erect herb or procumbent, annual or perennial herb of about 1-2 meter in height, main root long, cylindrical, thick, secondary **tertiary roots** present, slightly ribbed yellowish brown in colour; odour slight; taste slightly sweet and mucilaginous. **Stems** yellowish brown, erect, branched, cylindrical, hairy, solid, about 60 cm height. **Leaves** ovate – elliptic or obovate, alternate, petiolate, acute, entire softly pubescent above and usually white woolly beneath. **Flowers** greenish white, in small dense axillary heads or terminal spikes. Bract and bracteoles persisting, ending in a spine. **Seeds** subcylindric, truncate at the apex, rounded at the base, brown and shining.

Fig 3 ***Trachyspermum ammi*** **fruit**

3.1.3 Macroscopic observation of *Trachyspermum ammi Linn* fruit:

Botanical Name: *Trachyspermum ammi Linn* or *Carum copticum*

Common Name: Bishop's weed, Carom seed, ajowan, ajwain, omum

Family: Apiaceae or Umbelliferae

Trachyspermum ammi Linn is an erect, annual, 0.3-0.9 m. High, glabrous or minutely pubescent. Leaves rather distant, 2-3 pinnate, ultimate segments, 1.3-2.5 cm, all linear. Fruits, occurs mostly as entire cremocarps with pedicel attached or detached at the base and difid stylophod at the apex, broadly ovoid, 1.5-3 mm in length and 1.2-2.8 mm in width, yellowish green, dorsal surface, convex with 5 distinct longitudinal ridges in each mericarp, surface warty, commissural surface flat, showing 2 darker longitudinal bands representing the vittae. Odour aromatic, Taste At first slightly bitter becoming strongly pungent producing slight numbness to the tongue.

3.2 Microscopic Observation of plant materials:

3.2.1 Microscopic Observation by Transverse section of *Nardostachys jatamansi DC* rhizome.

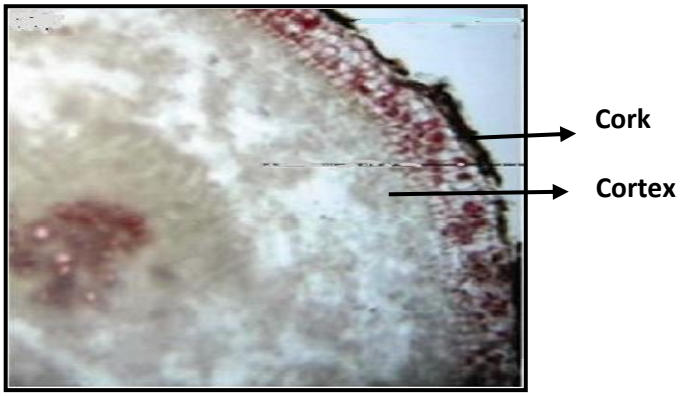


Fig.4 T.S. of *Nardostachys j atamansi* rhizome

Transverse section of rhizome is more or less circular in outline. Cork ,Cortex ,Phloem,Cambium ,xylem consists of vessels with pitted and scalariform thickenings. Medullary rays bi to multiserrate, not much prominent. Parenchymatous pith is present in the center. Starch grains abundantly present in groups.

3.2.2 Transverse section of *Achyranthus aspera Linn* whole plant:

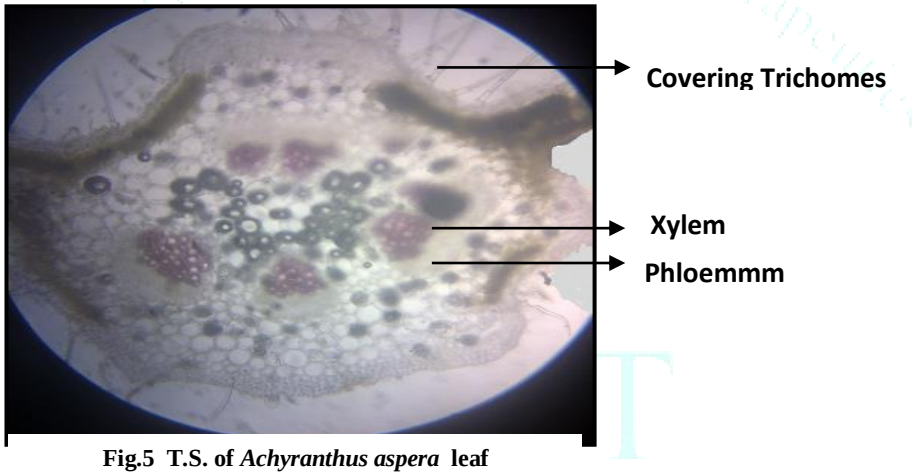


Fig.5 T.S. of *Achyranthus aspera* leaf

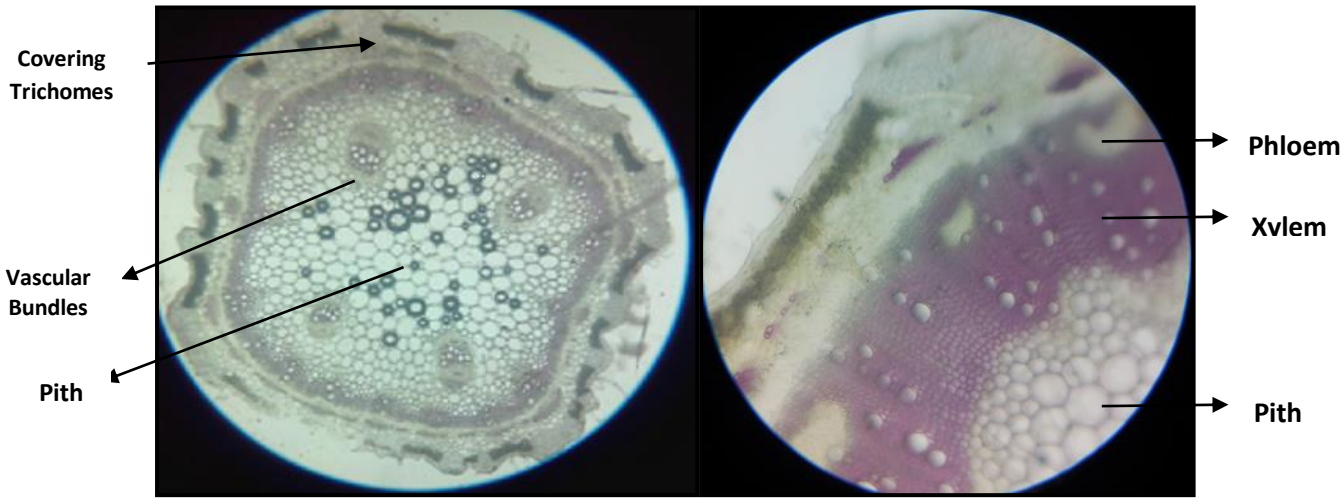


Fig.6 T.S. of *Achyranthus aspera* stem

Stem: Transverse section of stem shows 6-10 prominent ridges and collenchyma is present under each ridge. Pitted vessels, Tracheids, Calcium oxalate crystals of rosettes, prismatic or granular forms being present. Phloem fibres absent.

Leaf: Both the epidermis shows anomocytic type of stomata. Trichomes of covering glandular types are seen, the lower surface possessing greater number. Rosettes of calcium oxalate crystals distributed among the lower palisade layer.

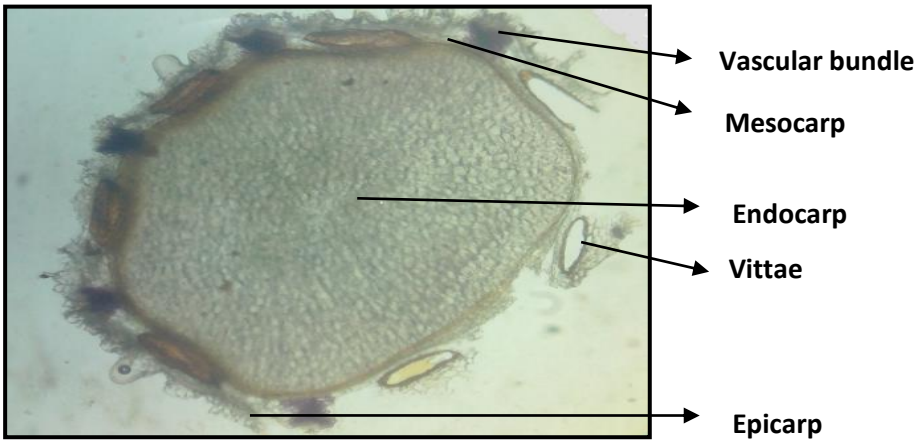


Fig.7 Transverse section of *Trachyspermum ammi* Linn fruit

2.3 Transverse section of *Trachyspermum ammi Linn* fruit.

Transverse section of fruit shows two hexagonal structures attached with each other's via a carpophores, 5 strongly developed primary ridges, each with a vascular bundles, 4 large vittae on the dorsal surface and 2 on the commissural surface, where lies raphae in between the endocarp and testa layer. Epicarp , Mesocarp , vittae, carpophores, vascular bundles ,Endocarp ,testa , endosperm were seen in section.

3.3 Table no. 1 shows the results of physicochemical analysis of three plants which complies with pharmacopoeia data.

Parameters	**Practical values**			**Values As Per IHP/Q.S**		
	N. J.	*A. A.*	*T. A.*	*N. J.*	*A. A.*	*T. A.*
Foreign matter	2.5%	1 %	1.5 %	NMT 5%	NMT 2%	NMT 2 %
Moisture content Loss on Drying	2 %	1.5 %	2.5 %	---	---	---
Ash value: Total ash	8 %	8.5 %	8 %	NMT 9 %	NMT 10 %	NMT 8.1 %
Water soluble ash	6.5 %	5 %	7 %	---	---	---
Acid insoluble ash	3 %	1 %	0.5 %	NMT 5 %	NMT 1.5 %	NMT 0.3 %
1) Extractive value: <u>Hot Percolation</u> Water soluble extractive	32 %	23 %	25 %	--- NLT 5 %	--- NLT 18 %	--- NLT 29 %
<u>Cold maceration</u> Water soluble extractive	30 %	20 %	20 %	NLT 2 %	NLT 4 %	NLT 20 %
2) Alcohol soluble extractive	20 %	7.5 %	25 %			
3) Ether soluble extractive	1.5%	2 %	28 %	---	---	---
Foaming Index	<100	>100	<100	---	---	---
Swelling Index	0.5 ml	6.0 ml	3.0 ml	---	---	---

NMT: Not more than NLT: Not less than

3.4 Phytochemical study:

3.4.1 Preliminary phytochemical screening of plant materials.

3.4.1.1 Preliminary phytochemical screening of *Nardostachys jatamansi DC* rhizome.

Table no.2 shows Different extracts of *Nardostachys jatamansi DC* rhizome with their appearance and yield

Sr. No.	Solvent		Color and Consistency	% Yield of Extract (w/w)
1.	Successive extract	Petroleum ether (60-80oc)	Brown (Sticky)	3.0 %
2.		Benzene	Brown (Sticky)	4.0 %
3.		Chloroform	Blackish Brown (Non-Sticky)	5.0 %
4.		Ethyl acetate	Light Brown (Non-Sticky)	2.8 %
5.		Methanol (95 %)	Reddish Brown (Non-Sticky)	3.0 %
6.		Water: Chloroform (99:1)	Brown (Non-Sticky)	5.5 %
7.	Hydro alcohol (50 %)		Dark Brown (Sticky)	14 %

3.4.1.2 Preliminary phytochemical screening of *Achyranthus aspera Linn* whole plant.

Table no.3 Different extracts of *Achyranthus aspera Linn* whole plant with their appearance and yield

Sr. No.	Solvent		Colour and Consistency	% Yield of Extract (w/w)
1.	Successive extract	Petroleum ether (60-80oC)	Green (Sticky)	4.0 %
2.		Benzene	Green (Non-Sticky)	1.0 %
3.		Chloroform	Green (Non-Sticky)	1.0 %
4.		Ethyl acetate	Light Brown (Non-Sticky)	3.0 %
5.		Methanol (95 %)	Brown (Sticky)	9.0 %
6.		Water: Chloroform (99:1)	Brown (Non-Sticky)	8.0 %
7.	Hydro alcohol (50 %)		Dark Brown (Sticky)	21.5 %

3.4.1.3 Preliminary phytochemical screening of *Trachyspermum ammi Linn* fruit.

Table no.4 Different extracts of *Trachyspermum ammi Linn* fruit with their appearance and yield

Sr. No.	Solvent		Color and Consistency	% Yield of Extract (w/w)
1.	Successive extract	Petroleum ether (60-80oC)	Green (Sticky)	30 %
2.		Benzene	Green (Sticky)	2.0 %
3.		Chloroform	Whitish Green (Sticky)	2.0 %
4.		Ethyl acetate	Light Green (Sticky)	1.8 %
5.		Methanol (95 %)	Greenish Brown (Sticky)	6.0 %
6.		Water: Chloroform (99:1)	Dark Brown (Non-Sticky)	14 %
7.	Hydro alcohol (50 %)		Dark Brown (Sticky)	24 %

3.4.2 Phytochemical analysis (Qualitative Chemical Tests) of different Successive extracts of plant materials

3.4.2.1 Phytochemical analysis of different extracts of rhizome of Nardostachys jatamansi (Qualitative Chemical Tests of Successive extracts) in below Table no.5

Test	**Successive Extracts**						Hydro alcohol
	PE	Benzene	Chloroform	Ethyl acetate	Methanol	Water	
Carbohydrates	--	--	--	--	+	+	+
Protein	--	--	--	--	--	+	+
Terpenoid/ steroid	+	--	--	--	+	--	+
Fats and oils	+	--	--	--	--	--	--
Glycoside	+	+	+	+	+	--	+
Alkaloids	--	+	+	+	+	+	+
Tannins/ Phenolic	--	--	--	--	+	+	+
Flavanoids	--	--	--	--	+	--	+

3.4.2.2 phytochemical analysis of different extracts of whole plant of Achyranthus aspera (Qualitative Chemical Tests of Successive extracts) in below Table no. 6

Test	**Successive Extracts**						Hydro alcohol
	PE	Benzene	Chloroform	Ethyl acetate	Methanol	Water	
Carbohydrates	_	_	_	_	+	+	+
Protein	_	_	_	_	+	_	+
Terpenoid/ steroid	_	+	+	+	+	_	+
Fats and oils	_	_	_	_	_	_	_
Glycoside	_	_	+	+	+	+	+
Alkaloids	_	_	+	+	+	_	+
Tannins/ Phenolic	_	_	_	_	+	+	+
Flavanoids	_	_	_	_	+	_	_

3.4.2.3 Phytochemical analysis of different extracts of fruit of Trachyspermum ammi (Qualitative Chemical Tests of Successive extracts) in below Table no. 7

Test	Successive Extracts						Hydro alcohol
	PE	Benzene	Chloroform	Ethyl acetate	Methanol	Water	
Carbohydrates	_	_	_	_	+	_	_
Protein	_	_	_	_	+	+	+
Terpenoid/ steroid	+	+	+	+	+	_	+
Fats and oils	+	_	_	_	+	_	+
Glycoside	+	_	_	+	+	+	+
Alkaloids	+	+	+	+	+	_	+
Tannins/ Phenolic	_	_	_	_	+	+	+
Flavanoids	_	_	_	_	+	_	_

Table shows yields of successive solvent extractions. Here higher yield achieved in alcohol and water extract. So 50% hydro alcoholic extract is performed for extraction and compared in phytochemical screening.

3.5 TLC and HPTLC profile of hydro alcoholic extract of Plant materials

3.5.1 TLC and HPTLC profile of hydro alcoholic extract of *Nardostachys jatamansi* rhizome

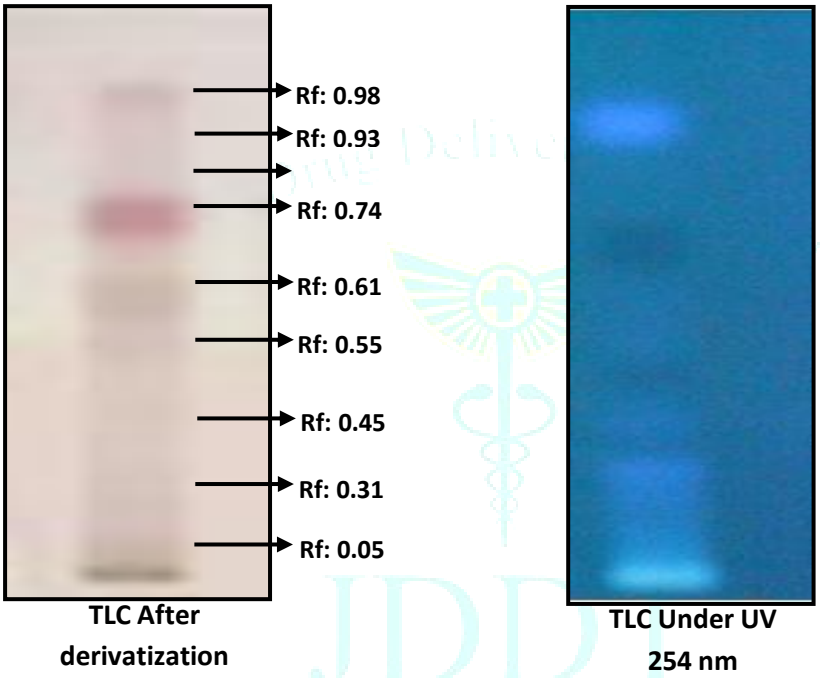


Fig. 8 TLC of hydroalcoholic extract of *Nardostachys jatamansi* rhizome

Peak no.	R_f	Peak Area
1	0.03	3651.53
2	0.35	466.59
3	0.48	658.57
4	0.57	578.35
5	0.74	600.82
6	0.81	456.17
7	0.93	795.88
8	0.98	371.52

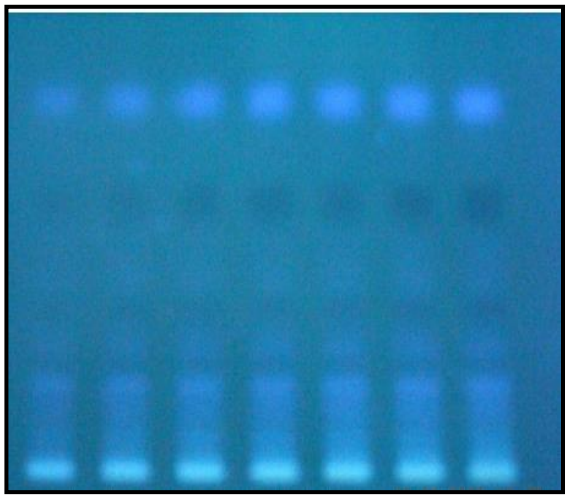


Fig.9 HPTLC fingerprinting of *N. jatamansi* rhizome extract

HPTLC fingerprinting of Hydro alcoholic Extract of ***Nardostachys jatamansi*** **rhizome** is shown in Fig.6 and their Rf values are shown in Table no. 8

3.5.2 TLC and HPTLC profile of hydro alcoholic extract of *Achyranthus aspera* plant

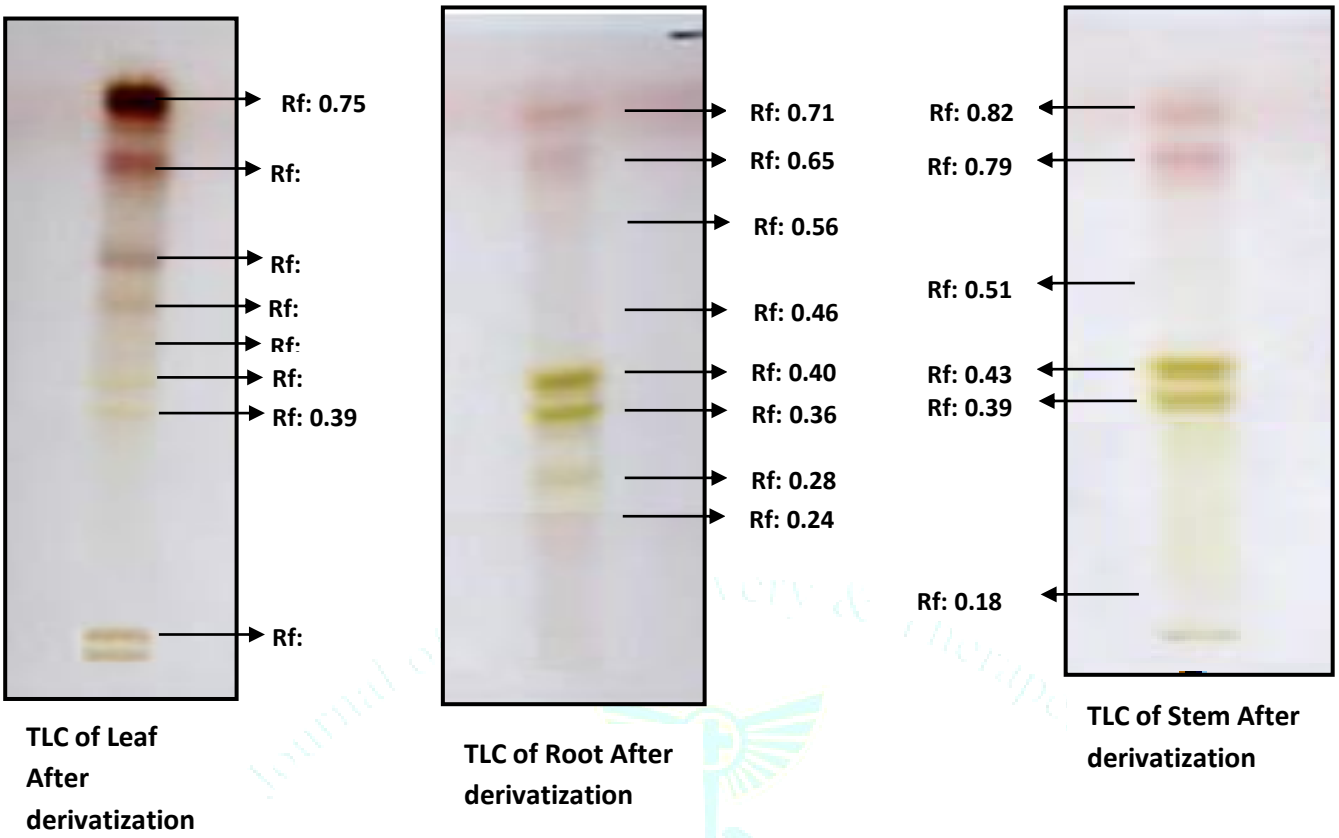
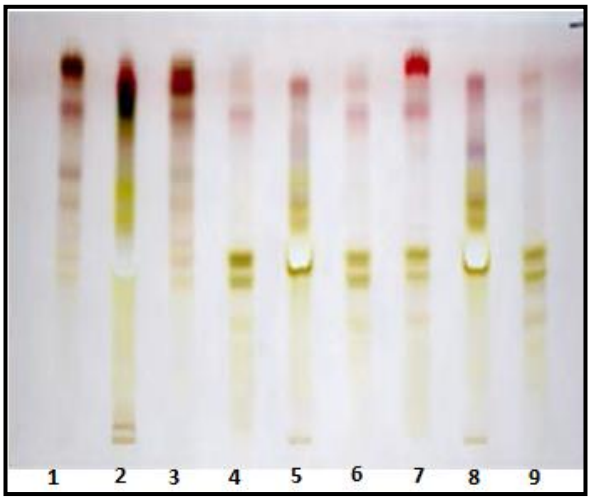


Fig. 10 TLC of hydroalcoholic extract of *Achyranthus aspera* plant

Table no.9 Reporting of HPTLC fingerprinting of hydro alcoholic extract of *Achyranthus aspera* plant

Peak no.	Rf value			Peak area		
	Leaf	Stem	Root	Leaf	Stem	Root
1	0.37	0.18	0.22	193.8	120.9	217.9
2	0.43	0.21	0.29	332.9	83.2	1453.5
3	0.46	0.28	0.38	219.7	618.0	3755.7
4	0.49	0.37	0.45	106.8	3489.5	3580.5
5	0.57	0.47	0.49	1508.5	6330.7	205.9
6	0.66	0.52	0.55	2929.1	116.7	261.5
7	0.79	0.78	0.68	3728.9	3906.8	134.0
8		0.84	0.79		990.6	1698.0
9			0.84			931.3



1-3: Leaf, 4-6: Stem, 7-9: Root

Fig.11 HPTLC Profile of leaf, stem and root of *Achyranthes aspera* in visible light

3.5.3 TLC and HPTLC profile of hydro alcoholic extract of Trachyspermum ammi fruit

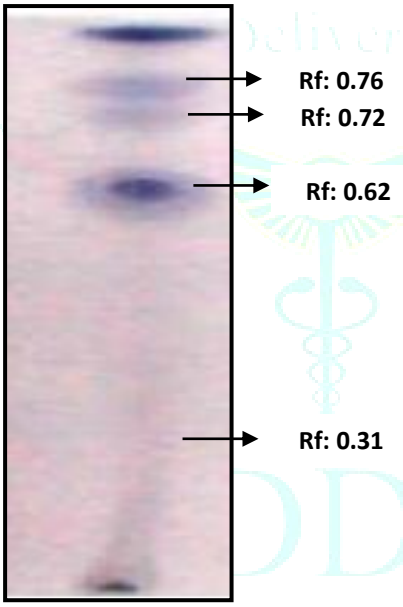


Fig. 12 TLC of hydroalcoholic extract of *Achyranthus aspera*

Table no.10 Reporting of HPTLC fingerprinting of hydro alcoholic extract of *Trachyspermum ammi* fruit

Peak no.	R_f	Peak Area
1	0.78	3728.9
2	0.70	2929.1
3	0.63	1508.5
4	0.48	332.9
5	0.31	219.7

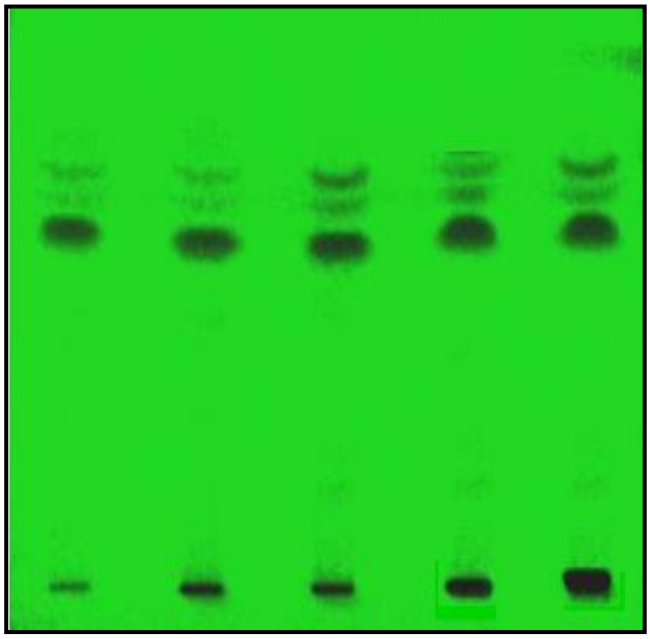


Fig. 13 HPTLC fingerprinting of *T. ammi* at UV 366 nm

4. SUMMARY AND CONCLUSION

The plant materials were identified by macroscopy and microscopy as described in Ayurvedic Pharmacopoeia. In microscopic observation, Physical parameters were found as per Ayurvedic Pharmacopoeia ranges for all plant components. Preliminary phytochemical screening of the extracts of three plants reveals that more yield and maximum constituents presents in Hydroalcoholic extract which showed the presence of Carbohydrate, Protein, Steroid, Glycosides, Alkaloids, Tannins and Flavanoids which may be responsible for their individual pharmacological activities. Primary TLC of Hydro alcoholic extracts of individual plant was done to identify and check major compound in Hydro alcoholic Extract. Also HPTLC supports the TLC profile by showing almost same bands for Hydroalcoholic extract which is useful for further study.

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