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PHARMACOGNOSTICAL, PHYTOCHEMICAL AND CHROMATOGRAPHIC FINGER PRINTING PROFILE OF PUSHKARMULA AND KUTH

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ABSTRACT

Background: The quality assessment of herbal drugs is of vital importance in order to ensure their acceptability in current era. **Objective:** In this study, two drugs (*Pushkarmula and Kuth*) mentioned in Ayurvedic literature were selected on the basis of their high therapeutic value (the roots are bitter, acrid, cardiotonic, thermogenic, expectorant, digestive; used in alleviates cardiovascular and respiratory complaints) and mentioned as critically endangered in IUCN (International Union for Conservation of nature) status. Because of above grounds, the economic value of drug is quite high and the chances of adulteration are also high. Collected (genuine) and market samples of *Pushkarmula (Inula racemosa* Hook. F.*)* and *Kuth (Saussurea lappa* C. B. Clarke*)* were collected from their natural habitat and raw samples of both drug from market of India respectively. **Material and Methods:** These samples were then subjected to Organoleptic, Pharmacognostical, Phytochemical and Chromatographical study (HPTLC and HPLC). **Result:** Collected sample of *Pushkarmula* has pleasant and camphoraceous odour while *Kuth* has strong and characterstic odour. Cells of medullary rays are polygonal to round in *Pushkarmula* whereas in *Kuth* they are radially elongated. HPTLC study of collected and market samples of *Pushkarmula* at 254 nm wavelength showed 3spots and 2 spots respectively. While in *Kuth* and its market sample at 220 nm wavelength have 7

spots and 8 spots respectively. HPLC study of both samples of *Pushkarmula* and *Kuth* having identical peaks with allantolactone and costunolide with slight variations respectively. **Conclusion:** Based on the above findings we can differentiate both samples from their market ones.

KEYWORDS: *Pushkarmula Kuth*, Pharmacognosy, Chromatography, *Inula racemosa, Saussurea lappa.*

INTRODUCTION

In the present study we have choose two most important and useful drugs in Ayurveda i.e. *Pushkarmula (Inula racemosa)* and *Kuth (Saussurea lappa)*. The selection of drugs is made due to its clinical importance in medical field like respiratory diseases, hepatobiliary diseases etc. and also these drugs are of enormous importance to the phyto-pharmaceutical industry. It is well known fact that today in the open market different plant parts are used in place of *Pushkarmula, Kuth, Katuki* etc. and like this so many other drugs are being adulterated and as such used by both physicians and pharmaceutical industries. Therefore quality assurance of medicinal plant starting materials is of paramount importance to offer predictable efficacy of the *Ayurvedic* formulations. Since both the industry and the individual physician use these plants in a dry form, therefore a method to assess their collectedness in the dry form is the need of hour.

However, these drugs are somewhat controversial and sometimes substituted by other drug viz. *Kuth (Saussurealappa)* is substituted by *Pushkarmula (Inularacemosa)* and so on. Even most of the experts do not clarify and identify both drugs and used frequently one in the place of other. Though both of drugs have many similar properties and also look like same. But it should be clarify that which one is *Pushkarmula* and which one is *Kuth*. So that we can got accurate result in treatment by given proper and accurate drugs. Therefore, keeping in view, the above drugs has been selected for present study.

HPTLC and HPLC is a chromatographic technique used to separate a mixture of compounds in analytical chemistry and biochemistry with the purpose of identifying, quantifying or purifying the individual components of the mixture. HPTLC is the improved method of TLC which utilizes the conventional technique of TLC in more optimized way. HPTLC takes place in high speed capillary flow range of the mobile phase. HPLC relies on pumps to pass a

pressurized liquid and a sample mixture through a column filled with a sorbent, leading to the separation of the sample components.

MATERIALS AND METHODS

1) Collection, identification and preparation of plant material

The roots of original sample of *Pushkarmula* and *Kuth* were collected separately from its natural habitat Auli, Dist. Chamoli, state Uttarakhand during October, 2014 and the market samples were procured from Khari Bowli, Katra Tobbaco market, Old Delhi, October, 2014. All the collected samples were authenticated at Botanical Survey of India, Dehradun, Uttarakhand by referring the deposited specimen[1] (Voucher specimen no.115603 and 115604) and market sample were identified by the experts of Department of Dravyaguna, Uttarakhand Ayurveda University, Rishikul Campus, Haridwar, State-Uttarakhand, India. Both collected samples were washed thoroughly in tap water, shade dried and then homogenized to fine powder and stored in airtight bottles.

2) Macroscopic study

Table 1: Comparative macroscopic features of collected sample of *Pushkarmula* and *Kuth* (Fig.-1).

S. No.	Appearance	*Pushkarmula* (Root)	*Kuth* (Root)
1.	Size	Up to 5-14 cm. long in length and 2-4 cm. in thickness.	Up to 7-15 cm. long in length and 2-4 cm. broad at the thickest part.
2.	Shape	Hard, stout, cylindrical, twisted and **gradually tapering tap** roots. External surface is rough, longitudinal wrinkled and a central pith with numerous small white dot like structures. Root bark is not very well developed and not easily removable from woody portion. It bears rootlet scars.	Woody, stout, fusiform, arched, **slightly twisted**. Thin roots are cylindrical in shape. Outer surface is rough, having longitudinal wrinkles with ridges running straight or spiral.
3.	Colour	Dark Khaki.	Saddle Brown.
4.	Odour	**Pleasant and camphoraceous**	**Strong and characteristic**.
5.	Taste	Bitter	In start little sweet then bitter. Aromatic.
6.	Fracture	Fracture- short and **uneven;** after breaking, cutting portion shows centrally located pith with numerous small yellowish-white dot like structures.	Fracture- **short and horny**; A transverse cutting portion shows a brownish white surface with **three distinct regions** i.e. periderm as thin outer ring followed by a woody portion with fine radical striation and a central pith region.

Table 2: Comparative macroscopic characters of *Pushkarmula* from its market sample (Fig.-2).

Sl. No.	Source	Appearance	Size	Colour	Odour	Taste	Fracture
1.	Collected	Stout, hard, cylindrical, carrot-like at the upper end twisted and gradually tapering at the end rough, having some whitish colored lenticels,longitudinally wrinkled.	5-14 cm. long in length and 2-3 cm. in thickness.	Dark Khaki with longitudinal striations.	Characteristic and camphoraceous.	Bitter.	Short and uneven.
2.	Market	Stout, hard, cylindrical, carrot-like at the upper end twisted and gradually tapering at the end rough, having some whitish coloured lenticels, longitudinally wrinkled.	3-8 cm. long in length and 1-2 cm. in thickness.	Dark Khaki colour with longitudinal striations.	Characteristic and camphoraceous.	Bitter.	Short and uneven.

Table 3: Comparative Macroscopic Characters of *Kuth* from its Market sample (Fig.-3)

Sl. No.	Source	Appearance	Size	Colour	Odour	Taste	Fracture
1.	Collected	Fusiform, conical and tapering, collapse in the centre having longitudinal wrinkles which anastomose and ridges running straight or spiral.	7-18 cm. long in length and 1.5-3 cm. in thickness.	Saddle Brown.	Strong and aromatic.	Start with little sweetness and then bitter.	Short and horny.
2.	Market	Straight, stout, unbranched, more or less round in shape having longitudinal ridges, lenticels, and some rootlet scars.	3-5 cm. long in length and 2-2.5 cm. in thickness.	Khaki colour	Mild bitter and Sweet	Slightly bitter.	Tough and mealy surface.

3) Microscopic Study[2]

Table No. 4: Comparative microscopic character of root of *Inula racemosa* and *Saussurea lappa* (Fig.-4, 6).

S. No.	Appearance	*Inula racemosa* (Root)	*Saussurea lappa* (Root)
1.	Phellem (cork)	scanty and made up of 2-6 layers of cells	thick and made up of 3-6 layers of cells
2.	Phelloderm (cortex)	scanty, 20-25 celled thick	Abundant. 40-50 cells thick.
3.	Secondary Phloem	Extensively made up of medullary rays which are 3-6 celled thick. Medullary rays are traversed by secretory canals.	traversed by medullary rays which are 3-5 cells thick. Medullary rays are closely associated with xylary tissue.
4.	Medullary rays	polygonal to rounded	radially elongated.
5.	Xylary elements	Arranged in radial rows. Polygonal to round. Xylem elements 21-54 µ in diameter. Scalariform reticulate and helical thickenings. Few with bordered pits.	Radial multiples, clusters, tangentially and isolated types. Polygonal to round. Xylary elements 32-108 µ diameters. Scalariform to reticulate thickenings. Bordered pits.

4) Powder microscopy

Powder microscopic inspection of medicinal plant materials is indispensable for the identification of broken or powdered materials; the specimen may have to be treated with chemical reagents. (Fig.-5, 7).

5) Physicochemical study[3]

5.1) Determination of foreign matter

Collected and market sample of plant material was weighed by the electronic monopan balance and a thin layer of sample was spreaded on a white colour sheet. By bull lens, the layer was examined for foreign matter. Foreign matter was separated and collected in to another paper. Plant material was recollected and weight again. Percentage of foreign matter in relation to the total quantity of plant material was calculated.

5.2) Determination of Moisture Content

Firstly empty petridish was kept in hot air oven for ½ hr to remove moisture. Initial wt. of petridish was noted. Then 5 gm drug powder was taken in three petridish for 3 individual readings. Petridish were kept in hot air oven at 105^0 C for 3 hours. Then the petridish were taken out and kept in desiccators till to reach at room temperature. Wt. of petridish was taken

and noted. Then again petridish were kept in oven and then in desiccator till the wt. become constant. Final weight of the petridish was noted.

5.3) Determination of Total Ash

Silica Crucible was cleaned, dried well, labeled with glass pencils and then weighed to constant weight. 5 gm of powdered drug sample put in the Silica crucible. The drug was spread evenly in to a thin layer. This crucible was placed in a muffle furnace and ignited at a temperature of 600°C for about 6 hrs or more until the ash was totally free from Carbon. The crucible containing the ash was allowed to be cooled in desiccators and subsequently weighed to constant weight. The percentage of ash with reference to the air dried drug was calculated.

5.4) Determination of Acid Insoluble Ash

Acid insoluble ash measure the amount of silica and siliceous earth. The ash obtained from above procedure was added to 25 ml of diluted HCl (6N) in a beaker. Then the mixture was heated at temperature of 70 to 80°C for 5 minutes. The mixture was filtered into a pre-weighed Silica Crucible with a Whatman's filter paper No 42, transfixed to a beaker. The glass beaker containing Ash and HCl mixture was then washed with boiling water three times and the water was also poured to the Silica Crucible. The Silica Crucible with residual Ash was then dried in the oven at 50°C for 2 hrs. Then it was allowed to be cooled in desiccators and subsequently weighed and the Acid Insoluble Ash was calculated. The procedure was repeated three times for each sample and the average value was calculated.

5.5) Determination of Water Soluble Ash

The Ash obtained from above procedure was mixed with 100 ml of distilled Water in a beaker and the mixture was heated at 70-80°C for 5 minutes. This mixture was poured into a pre-weighed Silica Crucible fitted with a Whatman's filter paper transfixed in a beaker. The mixture was filtered into the beaker. The Silica crucible with residual insoluble ash was dried at 50°C in oven and then allowed to be cooled in desiccator and subsequently weighed and the water soluble ash was calculated. The procedure was repeated three times for each sample and average value was calculated.

6) Phytochemical Examination[4]

Preparation of Test Sample

5 gm of drug samples were taken in crucibles. The crucibles were kept in muffle furnace at 550°C for nearly 6 hours. After 6 hours, the ash was removed from the muffle furnace. The ash was dissolve in 5ml of slightly acidic water and this solution was used for detecting the presence of mineral elements. (table25, 26).

Qualitative tests for various functional groups

The methods employed to isolate these substances are termed as extraction method. Crude extracts obtained from such extraction can be qualitatively tested to ascertain the presence of different types of components. The protocol for the phytochemical screening had been taken from the research Published article.[5]

7) Chromatography Study Preparation of extract

50 gm of powdered root of both samples were extracted with 250 ml methanol at the temperature between 60 and 65^0 C for 24 h by using soxhlet extractor separately. The solvent was evaporated by rotator vacuum evaporator to obtain viscous semisolid masses. The semi-dry methanolic crude extract was subjected to High Performance Thin Layer Chromatography (HPTLC) and High Performance Liquid Chromatography (HPLC).

7.1 HPTLC of *Pushkarmula*

Identification of isoalantolactone by HPTLC.

TLC plates: Precoated silica gel 60 F_{254} plates (E. Merck) of uniform thickness of 0.2 mm.

Solvent system: Toluene: Ethyl acetate (9.3:0.7).

Test solution: Extract about each 5g of freshly powdered root of collected and market samples of *Pushkarmula* with 150 ml *n*-hexane (5-7 h). Filter and remove the solvent under vacuum. Dissolve 2 mg of the residue in 1 ml of *n*-hexane.

Standard solution: Dissolve 0.1mg of isoalantolactone in 1 ml of *n*-hexane.

Procedure: Apply separately 4µl and 8µl each of the test solution of collected and market samples of *Pushkarmula* on a precoated silica gel 60 F_{254} TLC plate (E. Merck) of uniform

thickness of 0.2 mm. Develop the plate in the solvent system to a distance of 8cm, dry in a current of hot air and scan the plate in the TLC scanner at 560 nm.

Identification of isoalantolactone in the drug: Apply separately 4µl and 8µl each of test solution on a TLC plate. Develop the plate in the solvent system to a distance of 8cm. and record the chromatogram and Rf value (0.55) of isoalantolactone. The presence of isoalantolactone in the samples is determined by matching it with the standard.

7.2 HPTLC of *Kuth*

Identification of costunolide by HPTLC.

TLC plates: Precoated silica gel 60 F_{254} plates (E. Merck) of uniform thickness of 0.2 mm.

Solvent system: Toluene: Ethyl acetate (9.7:0.3).

Test solution: Extract about each 5g of freshly powdered root of Collected and market samples of *Kuth* with 50 ml petroleum ether (60-80^{0}C) by shaking for 30 min. at 50^0 in a conical flask. Filter and concentrate the filtrate under vacuum to about 25ml.

Standard solution: Dissolve 5 mg costunolide in 10 ml petroleum ether.

Procedure: Apply separately 4µl and 8µl each of the test solution of Collected and market samples of *Kuth* on a precoated silica gel 60 F_{254} TLC plate (E. Merck) of uniform thickness of 0.2 mm. Develop the plate in the solvent system to a distance of 8cm, dry in a current of hot air and scan the plate in the TLC scanner at 220 nm.

Identification of costunolide in the drug: Apply separately 4µl and 8µl each of test solution on a TLC plate. Develop the plate in the solvent system to a distance of 8cm. and record the chromatogram and Rfvalue (0.45) of costunolide. The presence of costunolide in the samples is determined by matching it with the standard.

7.3 HPLC of *Pushkarmula*[6],[7]

Collected sample was sent for HPLC study and the protocol for the study had been taken from the research Published article ‘Separation of Isoalantolactone and Alantolactone in *Inularacemosa*Root by RP-HPLC’ by Meena Sharma et al, Dabur Research and Development Centre, Dabur India Limited, Sahibabad, Ghaziabad (U.P.), India. Market sample of

Pushkarmula was also sent for HPLC study under same HPLC conditions as for *Inularacemosa*.

Sample

1) *Pushkarmula*

2) Market sample

Mobile Phase: Acetonitrile: 0.1 M Ammonium acetate in water (1:1).

Sample Preparation*:* A 5.0g amount root samples were extracted using methanol (100ml) on a sonicator for 4 hr. An aliquot of 1ml from the methanolic extract was filtered through a 0.45µ nylon syringe filter and analyzed.

Procedure: Separately inject 20 µl each of sample preparation of Collected and market sample of *Pushkarmula*. Record the chromatograms calculate the average area and finally calculate the percentage of alantolactone.

Apparatus: HPLC analysis was performed with a LC system consisting of a HP Agilent 1120 series isocrotic pump, and degasser. The samples were injected to a HP Agilent 1120 C_{18}column Inertsil ODS – 3V (250 mm X 4.6mm, particle size 5µm) at 30^{o} C. the system was controlled and data analysis was performed with Agilent EZChrom Elite software.

Chromatographic Conditions

Column	:	Inertsil ODS-3V (250 mm X 4.6mm, particle size 5µm)
Wavelength	:	254 nm
Flow rate	:	1 ml/min
Run time	:	25 min
Injection volume	:	20 µl

7.4 HPLC of *Kuth*

Collected sample was sent for HPLC study and the protocol for the study had been taken from the research Published article. Market sample of *Kuth* was also sent for HPLC study under same HPLC conditions as for *Saussurea lappa*.

Sample

1) Collected sample

2) Market Sample

Sample Preparation*:* Powdered sample extracted with methanol by sonication for 30 min. The obtained extracts were centrifuged at 4000 rpm for 20 min and the supernatant liquid was concentrated under vacuum at 40^{o}C. The extract was diluted with methanol in 33.3 mg/mL solution and injected directly on to HPLC system.

Mobile Phase: Acetonitrile and Water in 60:40, % v/v ratio, both solvents containing 0.1% Formic acid.

Apparatus: HPLC analysis was performed with a LC system consisting of a Agilent 1120 series isocrotic pump and degasser. The samples were injected to Agilent 1120, C_{18}column Inertsil ODS – 3V (250 mm X 4.6mm, particle size 5µm) at 30^{o} C. the system was controlled and data analysis was performed with Agilent EZChrom Elite software.

Chromatographic conditions

Column	:	Inertsil ODS-3V (250 x 4.6mm, 5µ)
Wavelength	:	210 nm
Flow rate	:	1 ml/min
Run time	:	20 min
Injection volume	:	20 Ml

RESULTS

***Pushkarmula* (Root)**	***Kuth* (Root)**
	
Cut surface shows two demarcation of yellowish brown internal and greyish brown external areas	Cut surface shows three regions outer brownish colour ring, periderm as thin blackish ringfollowed by a woddy portion with fine radical striations

Figure1: Comparative macroscopic features of collected *Pushkarmula* and *Kuth.*

Collected	Market
	

Figure 2: Comparative Macroscopic Characters of *Pushkarmula* and its market sample

Collected	Market
	

Figure 3: Comparative Macroscopic Characters of *Kuth* and its market sample.

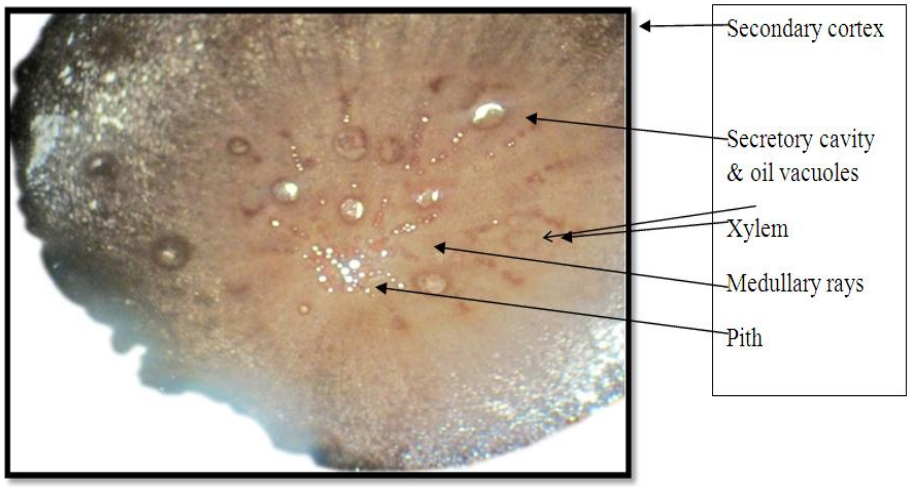


Figure No. 4: T.S. *Inularacemosa* Hook f.

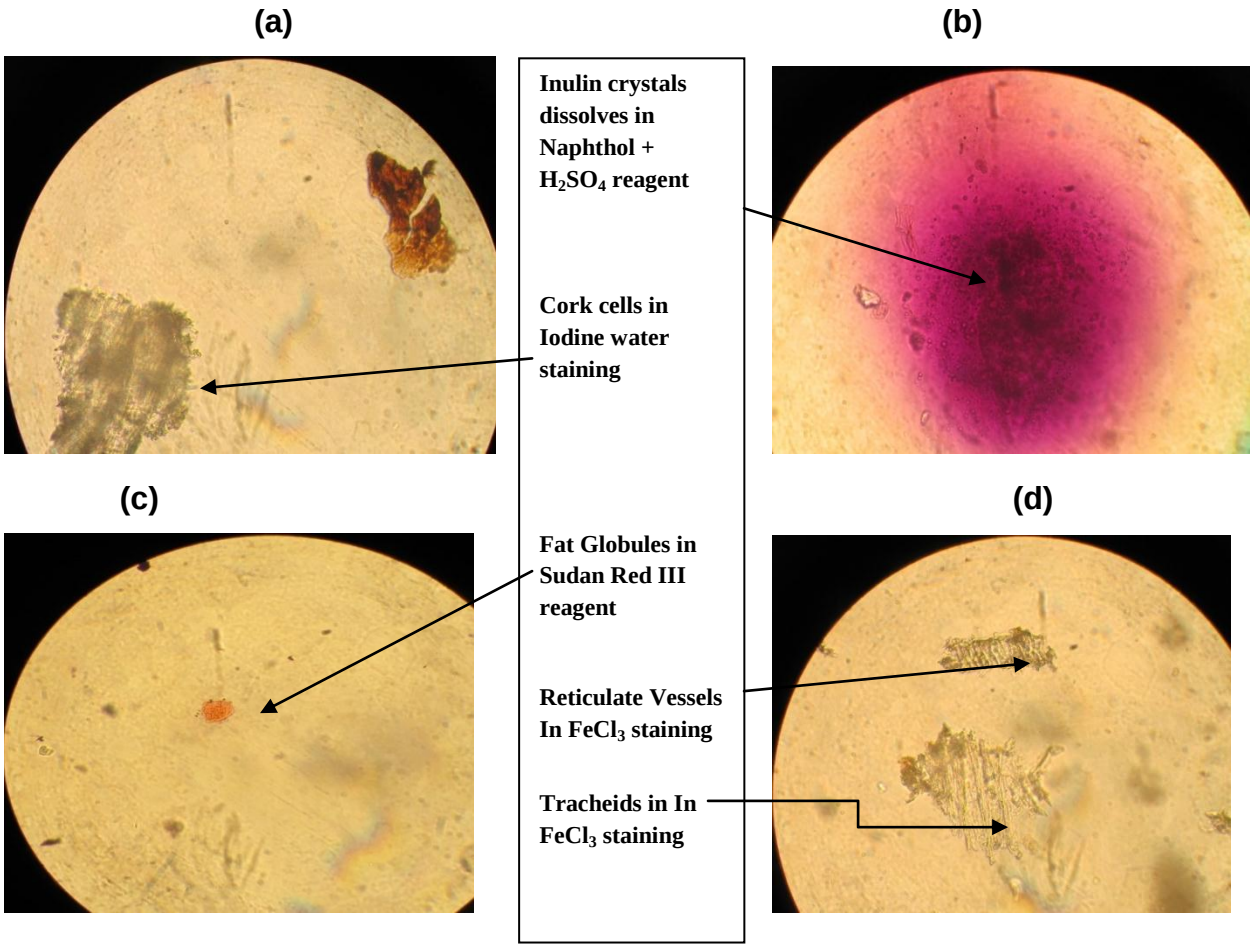


Figure No. 5: Powder Microscopy of *Inula racemosa* Hook f.

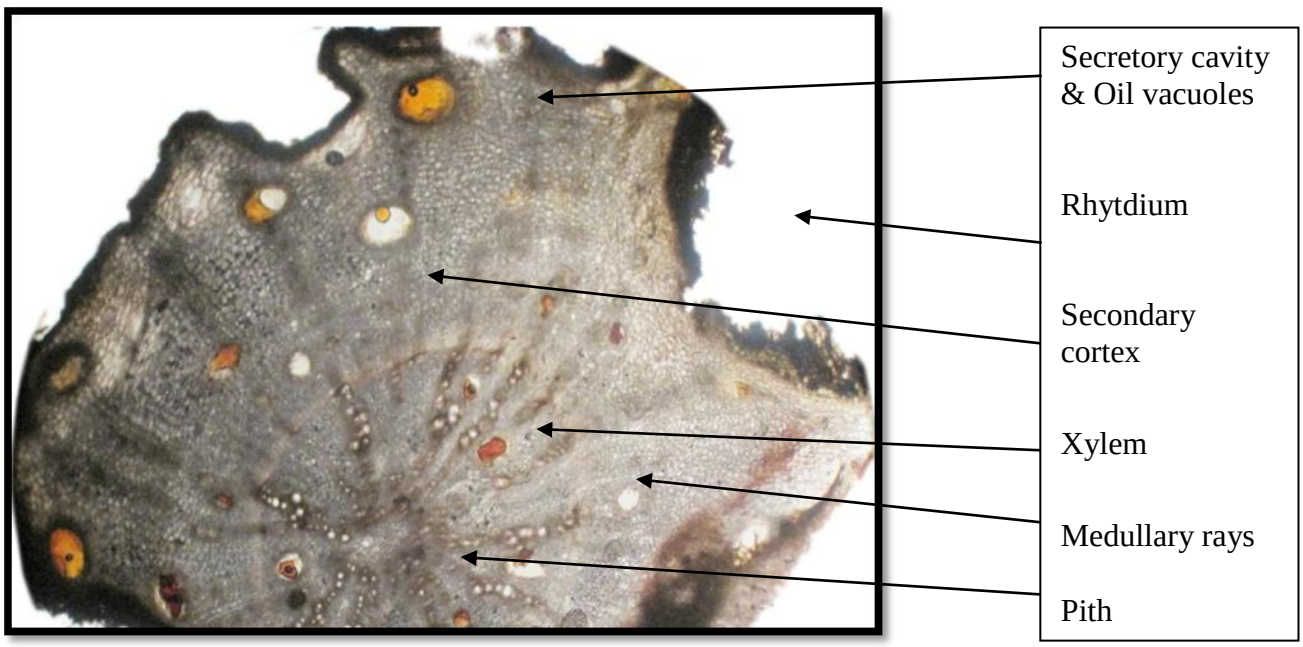


Figure No. 6: T.S of *Saussurea lappa* C.B. Clarke.

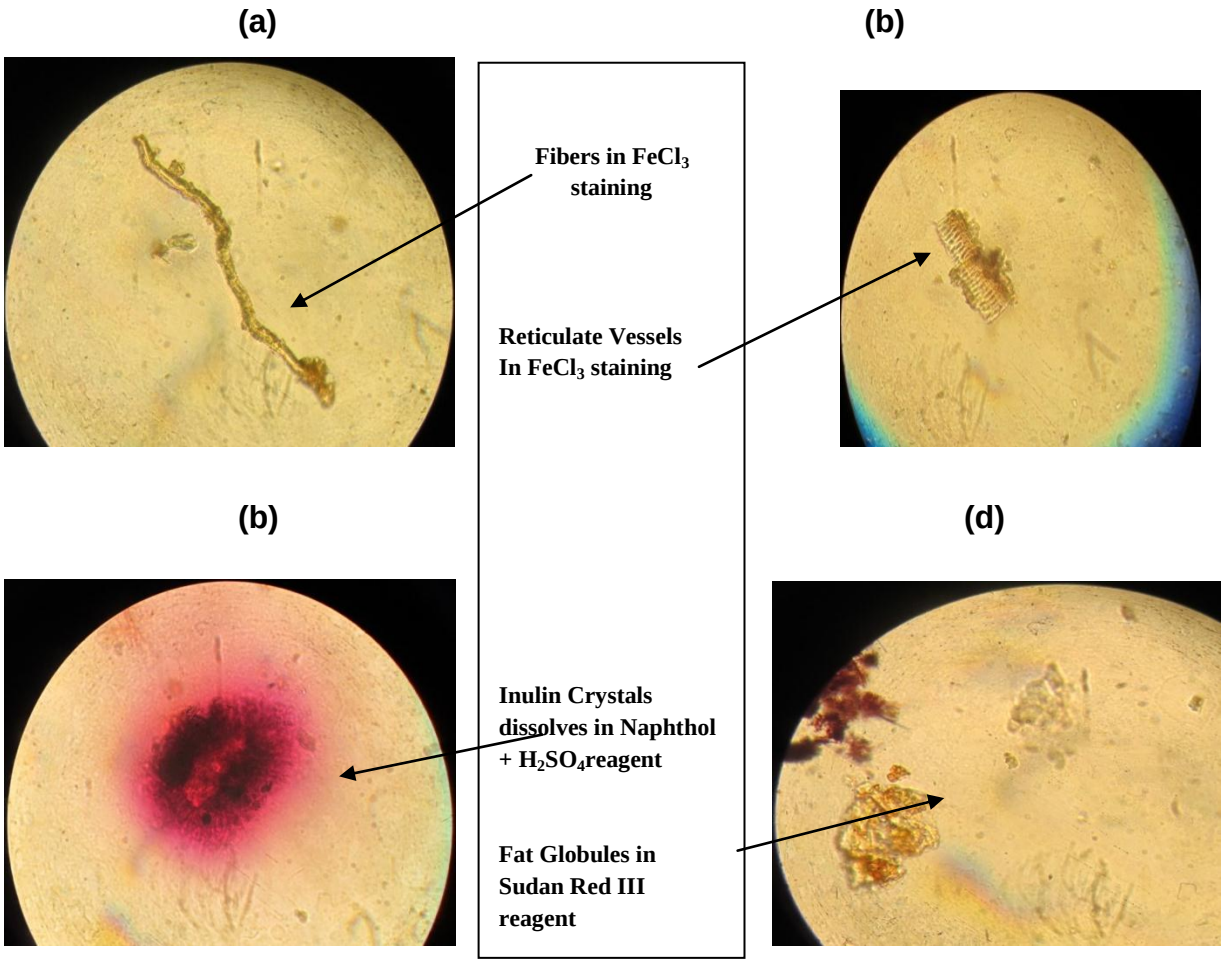


Figure No. 7 Powder Microscopy of *Saussurea lappa* C.B. Clarke

Table 5: Results of Staining of.

S. No	Chemical Reagents	*Pushkarmula*	*Kuth*
01	Safranin	Lignified cells present	Lignified cells present
02	Eosin	Cellulose and allurone grains absent	Cellulose and allurone grains absent
03	Ferric chloride	Tannin present	Tannin present
04	Naphthol	Inulin present	Inulin present
05	Iodine water	Starch absent	Starch absent
06	Sudan Red III	Cork cells present	Cork cells present

Table 6: Foreign matter of *Pushkarmula* and its market sample.

S.no	Sample	Total weight	Foreign matter	Percentage
01	Collected	500 gm	None	Zero %
02	Market	500 gm	2.90 gm	2.518 %

Standard- Not more than 2 % (API Part I Vol. IV Page no. 103).

Table 7: Foreign matter of *Kuth* and its market sample.

S.no	Sample	Total weight	Foreign matter	Percentage
01	Collected	500 gms	None	Zero %
02	Market	500 gms	6.909 gms	3.3818 %

Standard- Not more than 2 % (API Part I Vol. I Page no. 76)

Table 8: Moisture content of *Collected* and market sample.

S.No	Sample	Moisture Content	
		Pushkarmula	***Kuth***
01	Collected	14.86%	12.27%
02	Market	7.26%	6.47%

Table 9: Total ash content in Collected and its market sample.

S.No	Sample	Total Ash	
		Pushkarmula	***Kuth***
01	Collected	3.94 %	3.50 %
02	Market	6.78 %	7.69 %

***Pushkarmula*:** Standard- Not more than 5 % (API Part I Vol. IV Page no. 103).

***Kuth*:** Standard- Not more than 4 % (API Part I Vol. I Page no. 76).

Table 10: Determination of Acid insoluble and Water soluble ash

S.No	Sample	*Pushkarmula*		*Kuth*	
		A.I	**W.S**	**A.I**	**W.S**
01	Collected	0.38 %	0.24 %	0.89 %	0.20 %
02	Market	0.52 %	0.22 %	1.84 %	1.56 %

A.I: Acid insoluble, **W.S:** Water soluble

Pushkarmula: Standard- Acid insoluble not more than 0.6 % (API Part I Vol. IV Page no. 103).

Kuth: Standard- Acid insoluble not more than 1 % (API Part I Vol. I Page no. 76).

Table 11: Inorganic profile of the *Pushkarmula* and its market samples

S.No	Samples	Calcium	Iron	Magnesium	Phosphorus	Potassium	Sulphur
01	Collected	+ve	-ve	-ve	-ve	-ve	+ve
02	Market	+ve	-ve	-ve	-ve	-ve	+ve

Table 12: Inorganic profile of the *Kuth* and its market samples

S.No	Samples	Calcium	Iron	Magnesium	Phosphorus	Potassium	Sulphur
01	Collected	+ve	-ve	-ve	-ve	-ve	+ve
02	Market	+ve	-ve	+ve	+ve	+ve	+ve

(+ve): Presence of constituent (-ve): absence of constituent.

Table 13: Qualitative tests of *Pushkarmula* and its Market sample.

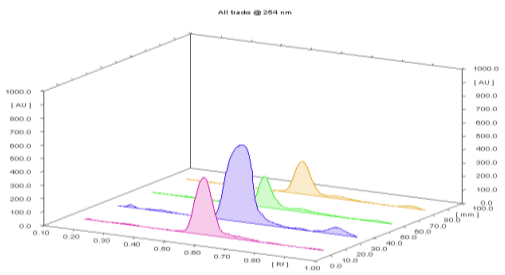
S. No.	Metabolites	Collected	Market
1	Carbohydrate	+ve	+ve
2	Proteins	+ve	+ve
3	Alkaloids	+ve	+ve
4	Tannins	-ve	-ve
5	Resins	+ve	+ve
6.	Quinones	-ve	-ve
7.	Saponin	+ve	+ve
8.	Steroids	+ve	+ve
9.	Coumarins	+ve	+ve
10.	Flavonoids	+ve	+ve
11.	Cardiac glycoside	+ve	+ve

Table 14: Qualitative tests of *Kuth* and its Market sample.

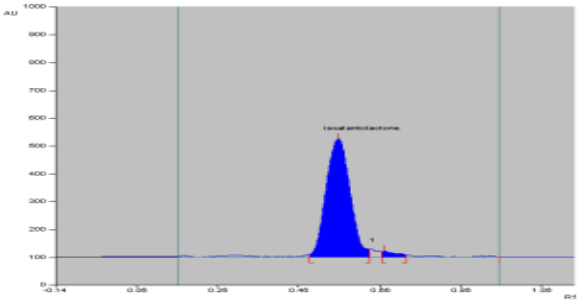
S.No	Metabolites	Collected	Market
1	Carbohydrate	+ve	+ve
2	Proteins	+ve	+ve
3	Alkaloids	+ve	-ve
4	Tannins	+ve	+ve
5	Resins	+ve	+ve
6.	Quinones	-ve	-ve
7.	Saponin	+ve	+ve
8.	Steroids	+ve	+ve
9.	Coumarins	+ve	+ve
10.	Flavonoids	+ve	-ve
11.	Cardiac glycoside	+ve	+ve

Identification of isoalantolactone in *Pushkarmula* extract by HPTLC

- Mobile phase: toluene: ethyl acetate(9.3:0.7)
- Scanning wavelength:254nm
- Spectra: 200-700 nm
- Mode: Absorption
- Rf: isoalantolactone 0.55
- Sample Identification(T1 & T2 –Market samples and T3 & T4-collected samples)



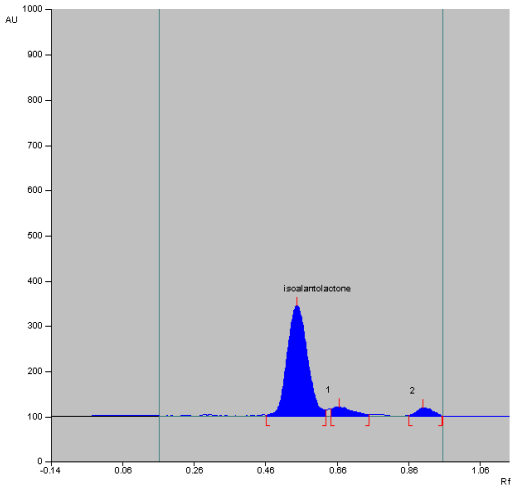
Peak Display\ 3-D display (Figure No. 8)



IDENTIFICATION OF ISOALANTOLACTONE IN ETHANOLIC EXTRACT OF PUSHKAR MULA MARKET SAMPLES (Figure No.: 9)

Table No. 15.

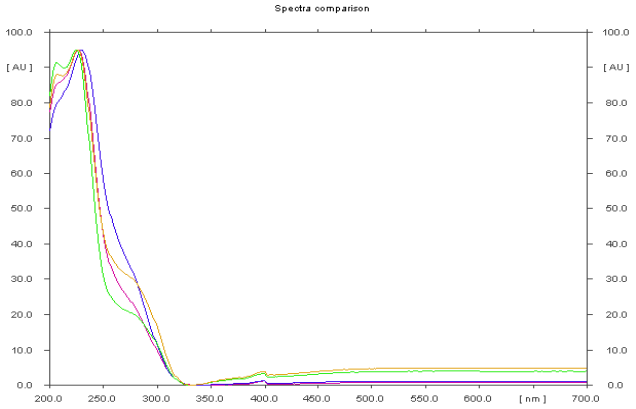
Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.48	9.7	0.56	425.1	95.00	0.63	28.5	20364.0	96.99	isoalantolactone
2	0.66	19.4	0.67	22.4	5.00	0.72	7.9	632.4	3.01	1



IDENTIFICATION OF ISOALANTOLACTONE IN ETHANOLIC EXTRACT OF *PUSHKAR MULA* COLLECTED SAMPLE (Figure No.- 10)

Table No. 16.

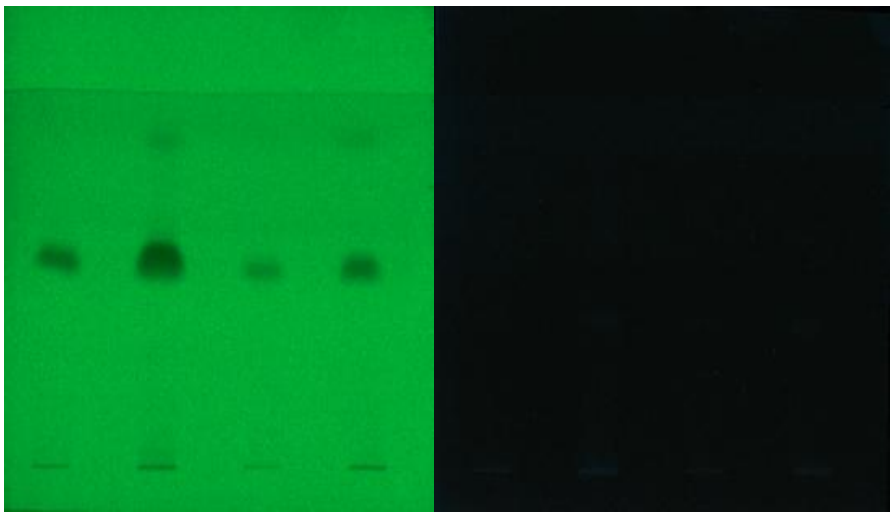
Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Ass
1	0.46	2.6	0.55	244.7	85.78	0.63	14.4	11214.2	86.82	isoalantolactone
2	0.64	15.5	0.66	21.1	7.39	0.75	4.1	989.1	7.66	1
3	0.86	2.1	0.90	19.5	6.83	0.95	0.4	712.7	5.52	2



Spectra of Isoalantolactone in Market and Collected sample (Figure No. 11)

RF of isoalantolactone in Market and Collected sample (Table No: 17)

Track	Vial	Rf	Height	X(calc)	Area	X(calc)	Sample ID/ Remark
1	1	0.56	425.05	425.055	20363.96	20363.957	PUSHKARAMULA MARKET SAMPLE
2	1	0.57	577.25	577.249	40808.43	40808.430	PUSHKARAMULA MARKET SAMPLE
3	2	0.53	228.83	228.831	8933.25	8933.253	PUSHKARAMULA GENUINE SAMPLE
4	2	0.55	244.73	244.733	11214.15	11214.153	PUSHKARAMULA GENUINE SAMPLE



T1 T2 T3 T4 T1 T2 T3 T4

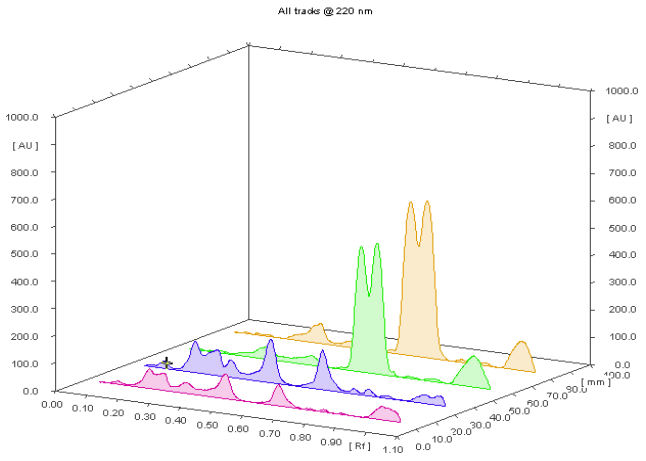
At 254 nm At 366 nm

PHOTO DOCUMENTATION RESULT (Figure No.- 12)

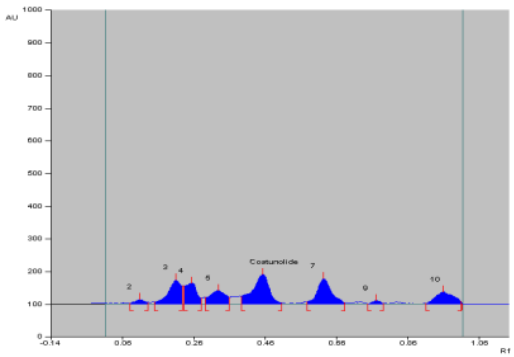
Identification of costunolide in *Kuth* extract

- Mobile phase: Toluene: ethyl acetate(9.7:0.3)
- Scanning wavelength:220nm
- Spectra : 200-700 nm
- Mode: Absorption

- Rf : Costunolide-0.45
- Sample Identification(T1 & T2 –Market samples and T3 & T4-Collected samples)



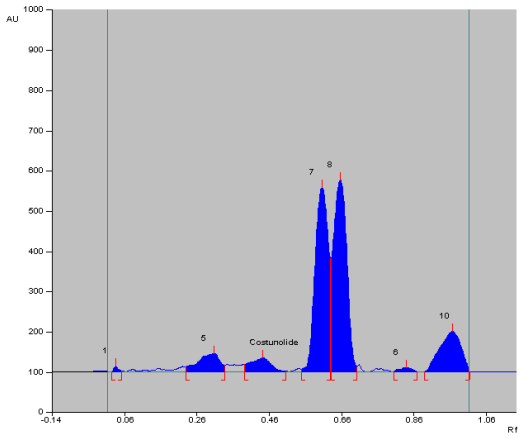
Peak Display\ 3-D display(Figure No. 13)



IDENTIFICATION OF COSTUNOLIDE IN ETHANOLIC EXTRACT OF *KUTH* IN MARKET SAMPLE (Figure No.-14)

Table No. 18.

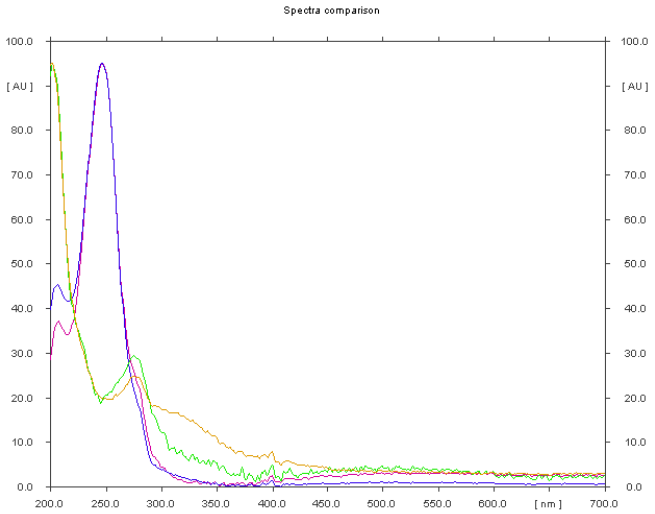
Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	
1	0.08	2.6	0.11	13.4	3.28	0.13	5.0	290.5	2.23	2
2	0.15	5.6	0.21	73.6	18.03	0.23	54.5	2058.7	15.79	3
3	0.23	54.9	0.25	64.1	15.72	0.28	18.3	1681.0	12.89	4
4	0.29	19.6	0.33	40.9	10.02	0.36	20.8	1454.5	11.16	5
5	0.39	23.5	0.45	90.1	22.09	0.50	3.0	3384.9	25.96	Costunolide
6	0.58	6.7	0.62	77.5	19.00	0.68	3.9	2480.5	19.03	7
7	0.75	0.1	0.77	10.5	2.58	0.79	2.2	179.2	1.37	9
8	0.91	1.1	0.96	37.9	9.29	1.01	7.4	1507.6	11.56	10



IDENTIFICATION OF COSTUNOLIDE IN ETHANOLIC EXTRACT OF *KUTH* IN COLLECTED SAMPLE (Figure No.-15).

Table 19.

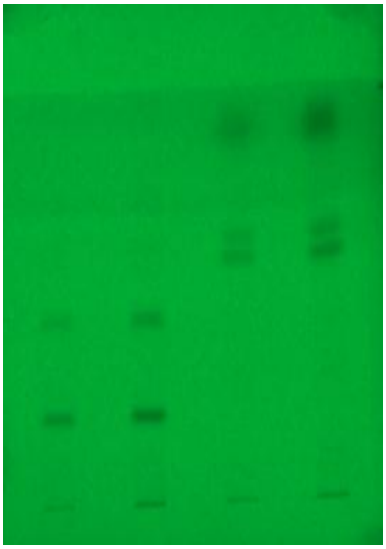
Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	
1	0.02	0.0	0.03	13.4	1.18	0.05	2.0	133.4	0.36	1
2	0.23	13.1	0.30	46.2	4.05	0.33	17.1	2207.0	5.98	5
3	0.39	18.6	0.44	35.0	3.07	0.50	1.0	1574.2	4.26	Costunolide
4	0.55	6.1	0.60	457.6	40.16	0.63	280.6	13584.5	36.80	7
5	0.63	281.0	0.65	476.8	41.84	0.70	13.3	14298.7	38.73	8
6	0.80	1.8	0.84	10.2	0.90	0.87	0.2	300.3	0.81	unknown *
7	0.89	0.1	0.96	100.3	8.80	1.01	4.0	4816.9	13.05	10



Spectra of Costunolide in Market sample & Collected Sample (Figure No. 16)

RF of Costunolide in Market sample & Collected Sample(Table-20).

Track	Vial	Rf	Area	X(calc)	Sample ID/ Remark
1	1	0.45	3384.89	3384.894	KUTHA MARKET SAMPLE
2	1	0.45	5412.37	5412.370	KUTHA MARKET SAMPLE
3	2	0.44	1574.15	1574.151	KUTHA GENUINE SAMPLE
4	2	0.45	2098.32	2098.324	KUTHA GENUINE SAMPLE



T1 T2 T3 T4 **T1 T2 T3 T4**

At 254 nm **At 366 nm**

PHOTO DOCUMENTATION RESULT (Figure No. 17)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Sepration of allantolactone from *Pushkarmula*

Chromatographic Conditions

Column	:	Inertsil ODS-3V (250 mm X 4.6mm, particle size 5µm)
Wavelength	:	254 nm
Flow rate	:	1 ml/min
Run time	:	25 min
Injection volume	:	20 µl

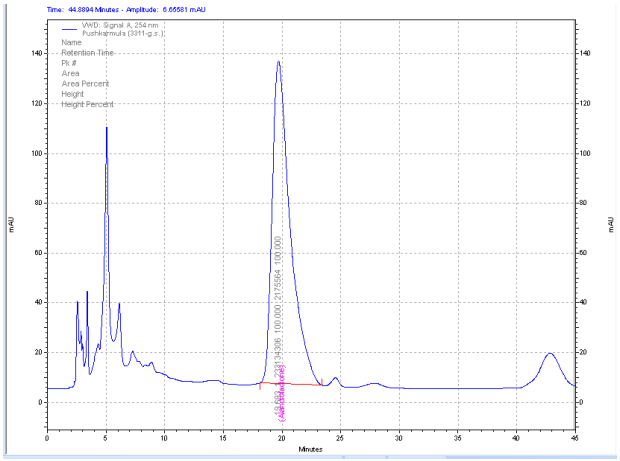


Figure No.18 *Pushkarmula* (Collected Sample)

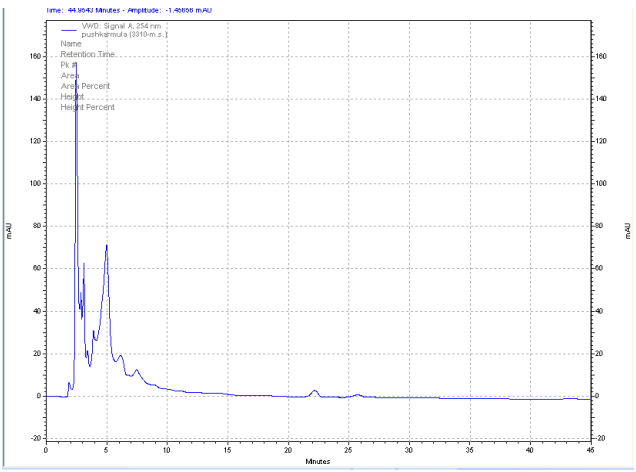


Figure No.19 *Pushkarmula* (Market Sample)

Results Table 21: [Fig. No. 18-19].

Peak	Name of Samples	Ret. Time	Area	Area %
	Pushkarmula (Collected sample)			
1	Alantolactone	19.683	233134306	100
	Pushkarmula (Market sample)			
1	Alantolactone	-	-	-

Sepration of costunolide

Chromatographic conditions

Column : Inertsil ODS-3V (250 x 4.6mm, 5µ)

Wavelength : 210 nm

Flow rate : 1 ml/min

Run time : 20 min

Injection volume : 20 µL

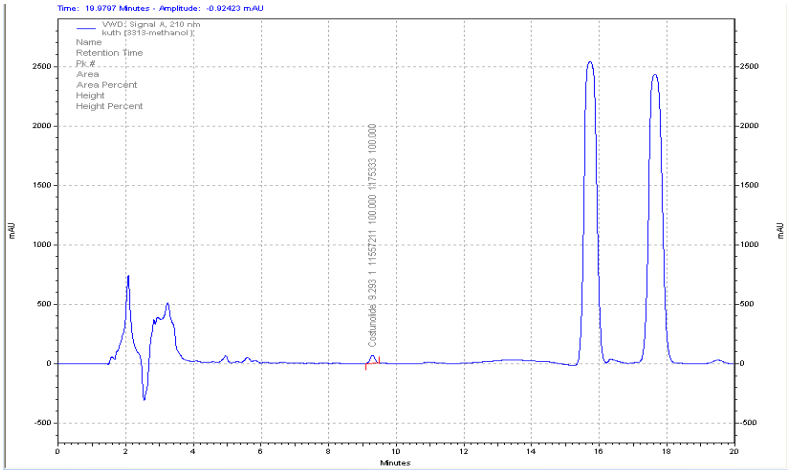


Figure No.20 *Kuth* (Collected Sample)

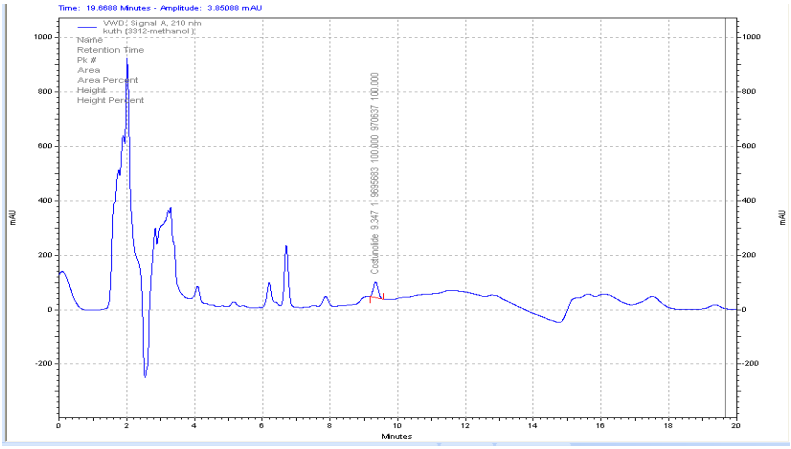


Figure No.21 *Kuth* (Market Sample)

Results Table 22: [Chromatogram No. 20-21].

Peak	Name of Samples	Ret. Time	Area	Area %
	Kuth (Collected sample)			
1	Costunolide	9.293	11557211	100
	Kuth (Market sample)			
1	Costunolide	9.347	9695683	100

DISCUSSION

After studying macroscopically both the samples of market has more than 50 % of the material was unmatured roots with stem and sold, *Pushkarmula* under the name of *Kaduwa Kuth* and *Kuth* under the name of *Meetha Kuth.* Microscopic study revealed that there was not much difference when compared both collected roots, the only difference was cells of the medullary rays are polygonal to round in *Pushkarmula* whereas in *Kuth* they are radially elongated.

Pharmacognostical study showed that collected samples of *Pushkarmula* and *Kuth* had 0% foreign matter whereas market samples had foreign matter over the standard limit. Amount of moisture content was more in collected sample than market. Both collected samples had total Ash value within the standard value but samples from market had 1 to 2% more than the normal values. Acid insoluble ash content in both samples of *Pushkarmula* under standard value while in *Kuth* market sample has. 84% more acid insoluble content. Water soluble ash of all the samples in increasing order collected then Market.

In phytochemical study the alcoholic extract of both *Pushkarmula* and *Kuth* showed the presence of Monosaccharides, Amino acid, Saponin, Glycosides, Alkaloides, Flavanoid, Steroids and Terpinoides are present in both samples collected and market. Reducing sugar present only in collected sample, whereas Tannins, Quinones and Phenols are absent in both sample.

Chromatographic Study revealed that High Performance thin layer chromatographic (HPTLC) of Alcoholic extracts of both samples of *Puskarmula* were developed using Toluene: Ethyl acetate in the ratio (9.3:0.7). After developing the fingerprints over a distance of 8 cm, the plates were dried and viewed under UV cabinet. After scanning by scanner 3 at 254 nm wavelength 3spots (Rf 0.53, 0.55, 0.90) were visible in collected sample and only 2 spots (Rf 0.56, 0.57) in market sample. Isoallantolactone and 1stunknown chemical constituent common in both samples. Whereas 2nd unknown constituent present in collected sample only which clearly distinguish both samples from each other. Whereas HPTLC of alcoholic extracts of *Kuth* and its market sample were developed using Toluene: Ethyl acetate in the ratio (9.7:0.3). After developing the fingerprints over a distance of 8 cm, the plates were dried and viewed under UV cabinet. After scanning by scanner 3 at 220nm wavelength 7 spots (0.03, 0.30, 0.44, 0.60, 0.65, 0.84, and 0.96) were visible in collected sample and 8 spots (0.11, 0.21, 0.25, 0.33, 0.45, 0.62, 0.77, and 0.96) in market sample. Costunolide, 5th and 10thunknown chemical constituents common in both samples.Whereas7th unknown constituent has higher concentration in collected than market.

8th unknown chemical constituent present in highest concentration in collected sample only which clearly distinguish both samples from each other. HPTLC finger prints can be used to identify *Pushkarmula* and *Kuth* from its adulterant which is sold in the market.

High Performance Liquid Chromatography (HPLC) of collected and Market sample of *Pushkarmula* was sent for HPLC study and the protocol for the study had been taken from the research Published article 'Separation of Isoalantolactone and Alantolactone in *Inula racemosa*Root by RP-HPLC' by Meena Sharma et al, Dabur Research and Development Centre, Dabur India Limited, Sahibabad, Ghaziabad (U.P.), India. The graphs obtained were compared with the published and identified peaks of allantolactone in the article. It was found that the collected sample of *Pushkarmula* were having identical peaks with the allantolactone with slight variations, whereas the market sample did not have allantolactone and not shows any peak with the graph of collected *Inularacemosa* sample (Table no. 5.5). So it clearly shows the difference in chromatographic profile between both samples.

Collected sample of *Kuth* and its market sample was sent for HPLC study and the protocol for the study had been taken from the Published research article by RNageswaraRaoet al; "HPLC determination of costunolide as a marker of *Saussurealappa* and its herbal formulations". Also during the market study market sample was also found to be sold under the name *Kuth* in the markets of Market. It is organoleptically and phytochemically different than original *Kuth* and also the HPLC conditions of market sample are available. Thus market sample was subjected to HPLC as per the published HPLC conditions. The graph obtained was compared with the published and identified peaks of costunolide in the article. It was found that the collected sample of *Kuth* was having identical peak with the costunolide with slight variation (Table 5.6). Market sample doesn't shows identical peaks. So it clearly shows the difference in chromatographic profile between both samples.

CONCLUSION

In present study, by pharmacognostical and phytochemical analysis we can 100% differentiate the roots of collected sample of both drugs, from its market sample also. During the market study, it was found that *Kuth* is being sold under two names *Meetha Kuth* and *Kaduwa Kuth*. Normally *Meetha Kuth* i.e. *Nagauri Ashwagandha* and other species of same plant is given when asked for *Kuth*, which is an entirely different plant and have no reference in texts for substituting *Kuth*, whereas substituting *Pushkarmula* in place of *Kuth* had many references in texts, which is sold under the name *Kadwa Kuth* in market. So in market Physician and researchers should ask for *Kadwa Kuth* while procuring *Kuth* or *Pushkarmula* both. but due to lack of knowledge traders sold mixture of *Kuth* and *Pushkarmula*. As per pharmacognostical view macroscopically root of *Kuth* on transverse cutting portion shows

three distinct regions while root of *Pushkarmula* only two. The odour of *Kuth* is stronger than *Pushkarmula*. In T.S. cell of medullary rays are polygonal to round in *Pushkarmula* whereas in *Kuth* they are radially elongated. The demand of *Pushkarmula* and *Kuth* has been increased remarkably in the last decade and also adulteration in all the samples had high amount of foreign matter mostly the unmatured roots and stem of the same plant. HPTLC profile of the root of both drugs reveals that the marker compounds resemble with root of collected samples of *I. racemosa* and *S.laapa* whereas market samples don't show identical peak with the respective standard peaks. HPLC reports showed that the absence of alantolactone in market sample of *Pushkarmula*. In the same way HPLC reports of both samples of *Kuth was* found that the Collected sample of *Kuth* were having identical peaks with the Costunolide with slight variations, whereas the market sample did not match with the graph of Collected sample. The reason behind the low standard of market samples to be the fact that the crude drugs have definite period of potency after which they lose their potency. Secondly both drugs occurring mountain ranges of Himalaya. It is collected there and is transported to other parts of the country before it is finally utilized in the preparation of therapeutic formulations. Time period between the collection and final utilization affects the therapeutic potential of crude drugs.

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