



PHYTO-PHARMACOGNOSTICAL STUDIES OF *JIVAK* [*CREPIDIUM ACUMINATUM* (D.DON)], *RISHBHAK* [*MALAXIS MUSCIFERA* (LINDL.) KUNTZE] AND THEIR MARKET SAMPLE

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Article Received on 11/04/2018

Article Revised on 01/05/2018

Article Accepted on 21/05/2018

ABSTRACT

Astavarga plants are renowned for their rejuvenating property from primordial time. First time “*Sharangadhara*” has quoted *Astavarga* term in formulation of *Astavarga churna*. *Jivak* and *Rishbhak* are described with their various formulations and indications in *Samhita* & *Chikitsa grantha*, having admiring properties like *Jivaniya* & *Vayasthapana* and play a major role in strengthening immune system to fight against diseases. Both the plants belong to “Orchidaceae” family. According to IUCN the conservation status of *Jivak* is in “Rare” category and of *Rishbhak* is in “Vulnerable” category. In present study, all aspects about *Jivak*, *Rishbhak* have been taken like name, geographical source, Macroscopy, Microscopy, Physicochemical, Phytochemical (Qualitative & Quantitative) & Chromatographic activities. For knowing current market status, market sample (S3) was procured in the name of authentic drugs. In Phytochemical study, on the basis of quantitative analysis Alkaloids, Bitter content, Saponins, Total Tannins, Flavonoids, Polyphenolic content were estimated. Total Tannins for *Jivak*-621.40mg/100gm, Flavonoid for *Rishbhak*- 143.45mg/100gm and Polyphenolic content for *Jivak*-241.10mg/100gm were maximum. A good percentage of Bitter content was found in market sample (S3) - 18.95%w/w. In Physicochemical analysis Aqueous extractive value of all these samples was maximum in comparison to Alcohol extractive value. All these parameters will help in confirming identity, quality, purity, potency, safety and efficacy of these plants.

KEYWORDS: *Jivak*, *Rishbhak*, Alkaloid, Saponin, Tannin, Polyphenolic content, Flavonoid.

INTRODUCTION

In Today era, extensive knowledge of medicinal plants become esoteric, confusing and on the edge of extinction. In older days transmission of knowledge was done as verbally from one generation to another. Documentation of knowledge was difficult task. Due to many obstacles such as lack of literature sources in different languages and missing histories of Ayurvedic text, there was depreciation of some valuable knowledge about medicinal herbs. *Jivak* and *Rishbhak* having admiring properties like *Jivaniya* & *Vayasthaphan* comparable to nutritive, anti-aging & anti-oxidant properties of modern pharmacology. In *Charak samhita*^[1] these two plants are included in *Jivanya mahakashya* and *Sukrajanana mahakashya*. *Jivak* also being a part of *Snehopag mahakashya*. In *Sushruta samhita*^[2] these 2 plants are included in *Kakoliyadi gana* & *Vidarigandhadi gana*. In *Astanga sangraha*.^[3] Acharya has mentioned these plants in the text of *Rasayana-vajikarana*, *Jivaniya Mahakasaya*, *Shukrajanan Mahakasaya*, *Padamkadi gana*,

Vidarigandhadi gana, *Jivan panchmool* and *Madhur skandh*.

Jivak and *Rishbhak* are counted in *Astavarga*. *Astavarga* is a group of eight medicinal plants (*Jivak*, *Rishbhak*, *Meda*, *Mahameda*, *Kakoli*, *Kshirkakoli*, *Ridhi*, *Vridhi*). *Ashwani kumar* has formulated a well known preparation named as *Chayvanprash* in which some plants of *Astavarga* are used. First time *Chayvan Rishi* used this preparation to renovate himself. In *Samhita* and *Chikitsa grantha* the importance of *Astvarga* plants has already mentioned so one should not get confuse regarding their medicinal effect. Now a days life style of people is very unsystematic. This haphazard lifestyle is a major cause of all diseases spreading around. These 8 plants are immunomodulator and play a major role in strengthening immune system to fight against diseases. In *Nighantus* both properties and synonyms of *Jivak* and *Rishbhak* have been given. A huge number of synonyms based on their morphological characters create a lot of confusion about their identification. Hence the need of the hour is

to establish parameters about their standardization. Also the present urgency is to show the importance of these plants which is helpful in their culturing & conservation.

Collection of Herbs

Collection of these plants from Uttarakhand region has done by the scholar himself because their biological place is already given in *Bhavprakash nighantu*^[4] as “*Himadri shikro udhbhav*” for *Jivak*, *Rishbhak*. It has been noticed in study that *Jivak* mostly exists in colonies, in sloppy shady areas at a altitude of 1200-2000m and it is easily expel out from the soil. *Rishbhak* is a high altitude plant of range 2500-4000m and has been seen scarcely at rainy areas adjacent to *Cotaneaster* species. Nearby its surrounding also *salam-mishri* (*Satyrium nepalense*) and *Liparis rostrata* are observed. *Liparis rostrata* is similar looking plant as *Rishbhak*, having identical leaf pattern and rhizome. Both plants differ in their inflorescence only.

For the research work scholar has also visited market place just to know what is presently commercially available in the name of these drugs and procured sample in the name of *Jivak -Rishbhak*. Pharmacognosy of these plants has been done so that no confusion will present regarding their identification.

1. *Jivak- Crepidium acuminatum* (D.Don)

Synonyms^[5]: *Crepidium acuminatum* (D.Don) Szlach Syn. *Malaxis acuminata* D.Don Syn. *Microstylis wallichii* Lindl.

Vernacular names^[6,7] – Sanskrit : *Jivakh*, Hindi : *Jivak*, English : *Jeevak*, Kannada : *Jivaka*, Telugu : *Jivakam*, Tamil : *Jivakam*, Gujarati : *Jivak*, Malayalam : *Jivakam*, China : *Quian lie zhao lan*, Vietnam : *Mao ail an nhon*.

Taxonomical Position^[8]

Kingdom : Plantae
Phylum : Tracheophyta
Class : Liliopsida
Order : Asparagales
Family : Orchidaceae
Subfamily : Epidendroideae
Genus : *Crepidium*
Species : *acuminatum*

Ayurvedic Properties^[9,10]

Rasa : *Madhura* (Sweet)
Guna : *Guru* (Heavy)
Virya : *Sheet* (Cold)
Vipaka : *Madhura* (Sweet)

2. *Rishbhak- Malaxis muscifera* (Lindl.) Kuntze

Synonyms^[11]: *Dienia muscifera* Lindl., *Microstylis muscifera* (Lindl.) Ridl.

Vernacular names^[12,13] – Hindi : *Rsabhak*, English : *Rishbhaka*, Kannada : *Rsabaka*, Tamil : *Itavagam*, Rsabaham, Guarati : *Rushabhak*, Bengali : *Rishbhak*,

Malayalam. : *Itavakam*, Nepali : *Rishbhak*, Sanskrit : *Rsabhakah*, *Vira*, *Indraksa*, *Visani*, *Durdhara*, *Vrsa*.

Taxonomical Position^[14]

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Phylum : Tracheophyta
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Family : Orchidaceae
Genus : *Malaxis*
Species : *muscifera*

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MATERIAL AND METHODS

Nowadays adulteration of crude herbal drugs is very common due to scarcity of drug and its high price prevailing in the market. These herbal medicines can stand in commercial market only if they are evaluated according to modern science. Evaluation of herb involves confirmation of its identity, determination of its quality and purity, and detection of nature of adulteration. So before utilizing a drug for therapeutic purpose, detail pharmacognostical study is essential as it not only help in correct identification of the drug but also to get a clue for its phytochemicals, pharmacological activities and medicinal properties.

Jivak, *Rishbhak* are valuable medicinal plants. They are important ingredients of *Chavyanprash*. Their other formulations are *Jivanya ghrta*, *Mahamayur ghrta*, *Vajikarana ghrta*, *Chitrakadi tail*, *Vachadi tail*, *Maha-Padma tail*, *Astavarga churna*, *Mahakalyan ghrta* etc. The formulations of these plants are commercially manufactured in huge amount, so standardization of these plants is necessary to ruled out adulteration and substitution. As the potency of these drugs for therapeutic purpose is proved by standardization only.

Material

The plant materials taken for the study were :

- Genuine and market samples of *Jivak- Crepidium acuminatum* (D.Don) Szlach. Syn. *Malaxis acuminata* D.Don
- Genuine and market samples of *Rishbhak* : *Malaxis muscifera*

Collection of genuine sample

- The genuine sample of *Jivak* i.e. *Crepidium acuminatum* (D.Don) Szlach. has collected from hills of Chakrata District- Dehradun, on the Way from Kunjapuri to Fakot and on the Way from Chamba to Dhanaulti, district - Tehri Garhwal, State - Uttarakhand.

Date of Collection: 12-07-2017

Geographical details of Habitat

- **Chakrata, Uttarakhand** (92 km from Dehradun)- At an altitude of 6,500 feet Approx. above sea level.
- **On the Way from Kunjapuri to Fakot** (38 km from Rishikesh)
- **On the Way from Chamba to Dhanaulti** (94 km from Rishikesh) - At an altitude of 4500 feet Approx. above sea level.
- The genuine sample of *Rishbhak* i.e. *Malaxis muscifera* has collected from Valley of flower, district- Chamoli, Deoban, District-Dehradun, and Chopta district - Rudraprayag, State – Uttarakhand.

Date of Collection: 20-07-2017

Geographical details of Habitat

- **Valley of flower, Uttarakhand** 15 km from Govind Ghat) - At an altitude of 11,600 feet Approx. above sea level.
- **Deoban, Uttarakhand** (97 km from Dehradun) - At an altitude of 9,500 feet Approx. above sea level.

- **Chopta, Uttarakhand** (24 km from Rudraprayag) - At an altitude of 8,790 feet Approx. above sea level.

Collection of Market Sample

Due to extensive industrialization and urbanization it has become almost impractical for Ayurvedic physicians to personally procure the authentic drugs and therefore totally depends on raw drug sellers and the middle men for procurement of medicinal plant raw materials. This exclusive dependence on traders has created serious malpractice of adulteration and selling of substandard medicinal plant raw materials in the market. So it is mandatory to study the market samples to check the adulteration. For the market study, one main market has selected from all over India and markets samples have collected by scholar himself. Following points were kept in mind while collecting market samples.

- ❖ Markets samples were collected as such and not verified on spot.
- ❖ Sample purchased has properly labeled, stored and subjected to investigation.

Details of Market Samples

S.No.	Markets	Date of Purchasing or receiving	Collector	Price of the sample
1.	Delhi	16/09/2017	Scholar	<i>Jivak-Rishbhak</i> : Rs.400/-Kg

Other Materials

- Magnifying Lens, measuring tape for organoleptic study.
- Safranin, Eosin, Ferric chloride, Naphthol, Iodine, Sudan red III and Ethanol for staining and Canon digital camera attached to Zeiss microscope was used for the microscopic study.

Methods**Herbarium and authentication of collected genuine plant material**

S.No.	Name of the plant	Time of Collection	Place of Collection	Authentication of Herbarium
1.	<i>Jivak</i> : <i>Crepidium acuminatum</i> (D.Don) Szlach.	June- July, 2017	Way from Chamba to Dhanaulti, district:Tehri Garhwal, Chakrata, Distt.:Dehradun	BSI, Dehradun
2.	<i>Rishbhak</i> : <i>Malaxis muscifera</i> (Lindl.) Kuntze	July, 2017	Valley of flower, district:Chamoli, Deoban(Chakrata), District:Dehradun, and Tungnath (Chopta) district:Rudraprayag	BSI, Dehradun

Table No.: Labeling of all Samples

Genuine sample of <i>Jivak</i>	S1
Genuine sample of <i>Rishbhak</i>	S2
Sample of Delhi <i>Jivak- Rishbhak</i>	S3

OBSERVATION AND RESULTS

Pharmacognostical Study

Macroscopy

Features	<i>Crepidium acuminatum</i> (D.Don)	<i>Malaxis muscifera</i> (Lindl.)
Habitat	Temperate to alpine zones of the Himalaya. In India, found in Utrakhhand, Himachal Pradesh etc.(North west Himalayas).	Temperate to alpine zones of the Himalaya. In India, found in Utrakhhand, Himachal Pradesh etc.(North west Himalayas).
Habit	Growing in sloppy, shaddy areas.	Growing in sloppy, shaddy, rainy areas.
Stem	5-7 cm long, tender, underground.	Long, erect, slender.
Leaves	Usually 2-4, sessile, 6-12 cm long, ovate-lanceolate, acute with prominent vein	1 or 2, unequal, sessile, 4-10 cm long, 2-5 cm broad, ovate to ovate-lanceolate, oblong.
Flowers	Small, short stalked, 8-10 mm in diameter.	Minute, 3-4 mm long, pale-yellowish green.
Pseudobulb	3-8 cm long, 1-3 cm in diameter, conical like a garlic clove, greenish covered with white papery sheath.	1-3 cm long, 0.5-1 cm in diameter, rounded like garlic or bull horn shaped, greenish covered with white-yellowish papery bark.

1. Organoleptic Study- Comparative Organoleptic features of *Jivak*, *Rishbhak* & Market sample

Character	Pseudobulb of <i>Jivak</i> <i>Crepidium acuminatum</i> (D.Don) Schlach.	Bulb of <i>Rishbhak</i> <i>Malaxis.muscifera</i> (Lindl.) Kuntze	Market sample of (<i>Jivak</i> - <i>Rishbhak</i>)
Shape	Conical	Rounded	Conical
Size	3-8 cm long, 1-3 cm in diameter.	1-3 cm long, 0.5-1 cm in diameter.	2-8 cm long, 1-3 cm in diameter.
Surface	Smooth, Shining & Fleshy,	Smooth, Shining, Fleshy,	Smooth, Shining & Fleshy,
Odour	Odourless	Odourless	Odourless
Colour	Green, covered with white papery membranous sheath.	Green, covered with white-yellowish papery membranous sheath.	Green, covered with white papery membranous sheath.
Taste	Mild sweet	Mild Sweet	Mild sweet
Fracture	Smooth	Smooth	Smooth

1. Transverse section microscopic study

Comparison of the features seen in the Transverse section of *Jivak* *Rishbhak* & Market sample

Characters	Genuine Sample of <i>Jivak</i>	Genuine Sample of <i>Rishbhak</i>	Market Sample of <i>Jivak</i> - <i>Rishbhak</i>
Epidermis	Single layer, thick wall.	Tubular epidermis with thin layer cuticle.	Single layer, thick walled.
Cortex	Irregular parenchyma cells with large air spaces.	Parenchymal cell with wide air spaces.	Irregular parenchyma cells with large air spaces.
Vascular bundle	scattered throughout the ground tissue surrounded by thick walled Bundle sheath (sclerenchymatous cells).	scattered throughout the ground tissue surrounded by thick walled Bundle sheath (sclerenchymatous cells).	scattered throughout the ground tissue surrounded by Bundle sheath (sclerenchymatous cells).
Phloem	Well developed	narrow zone of phloem	Well developed
Xylem	Well developed	Narrow zone of xylem	Well developed

1. Powder Microscopy Study

Comparison of the features seen in the Powder microscopy.

Features	<i>Jivak</i>	<i>Rishbhak</i>	Market sample
Cork cell	+	+	+
Xylem vessel	+	+	+
Stone cell	-	+	-
Starch grain	+	+	+
Annular Ring	+	-	+
Parenchyma cells	+	+	+
Scleroid cell	+	+	+
Lignified cell	+	-	+
Mucilaginous cell	-	+	-

1. Physicochemical Study

Test	Result			
	S1	S2	S3	API S1
Foreign matter	0 %	0%	1.34%	NMT 2%
Moisture content	9.72%	5.98%	5.63%	-
pH	5.18	4.57	5.69	-
Total Ash	2.96%	2.48%	2.31%	NMT 3%
Acid Insoluble Ash	0.42%	0.49%	1.2%	NMT 0.5%
Water Soluble Ash	1.42%	3.42%	0.95%	-
Starch content	1.42%	3.42%	0.95%	NLT 19%
Alcohol Extractive Value	7.81%	8.32%	6.02%	NLT 4%
Aqueous Extractive Value	18.27%	32.18%	15.05%	NLT 12%

2. Phytochemical Study

Qualitative Analysis

A. Carbohydrate test for different samples

Name of test	Aqueous extract	Alcohol extract	Sample
Fehling Test	+ve	-ve	S1
	+ve	-ve	S2
	+ve	-ve	S3

B. Alkaloid analysis in different samples

Name of test	Aqueous extract	Alcohol extract	Sample
Mayer Reagent	+ve	+ve	S1
	+ve	+ve	S2
	+ve	+ve	S3

C. Analysis of Proteins in different samples

Name of test	Aqueous extract	Alcohol extract	Sample
Biuret Test	-ve	-ve	S1
	-ve	-ve	S2
	-ve	-ve	S3

D. Analysis of Saponin in different Samples

Name of test	Aqueous extract	Alcohol extract	Sample
Foam Test	+ve	-ve	S1
	+ve	-ve	S2
	+ve	-ve	S3

E. Analysis of Glycosides in different samples

Name of test	Aqueous extract	Alcohol extract	Sample
Borntrager's Test	+ve	-ve	S1
	+ve	-ve	S2
	+ve	-ve	S3

F. Analysis of Phenolic compounds in different samples

Name of test	Aqueous extract	Alcohol extract	Sample
Phenolic Test	+ve	+ve	S1
	+ve	+ve	S2
	+ve	+ve	S3

G. Analysis of Steroids in different samples

Name of test	Aqueous extract	Alcohol extract	Sample
Salkowski Reaction	+ve	+ve	S1
	+ve	+ve	S2
	+ve	+ve	S3

H. Analysis of Tannin in different samples

Name of test	Aqueous extract	Alcohol extract	Sample
Ferric Chloride Test	+ve	+ve	S1
	+ve	+ve	S2
	+ve	+ve	S3

I. Analysis of Starch in different samples

Name of test	Aqueous extract	Alcohol extract	Sample
Iodine Test	+ve	-ve	S1
	+ve	-ve	S2
	+ve	-ve	S3

J. Analysis of Flavonoid in different samples

Name of test	Aqueous extract	Alcohol extract	Sample
Shinods Test	+ve	-ve	S1
	+ve	-ve	S2
	+ve	+ve	S3

K. Analysis of Terpenoid in different samples

Name of test	Aqueous extract	Alcohol extract	Sample
Terpenoid Test	+ve	+ve	S1
	+ve	+ve	S2
	-ve	+ve	S3

Quantitative Analysis**A. Quantitative Analysis of Jivak, Rishbhak & Market sample**

Quantitative value	(S1) %w/w	(S2) %w/w	(S3) %w/w
Alkaloids	0.98	0.90	0.59
Bitter content	15.09	12.38	18.95
Saponins	14.94	12.93	11.36

Quantitative value	(S1) mg/100gm.	(S2) mg/100gm.	(S3) mg/100gm.
Total Tannins	621.40	549.54	413.79
Flavonoid	124.78	143.45	112.39
Polyphenolic content	241.10	168.74	159.02

1. Chromatographic Study**TLC (Thin Layer Chromatography) analysis for different samples**

Solvent system: toluene : ethyl acetate (90 : 10) - Jivak, Rishbhak.

Sample: Ethanol Extract

Visualization: On spraying with *anisaldehyde sulphuric acid reagent* and heating the plate for 10 minutes at 105° spots. (Jivak, Rishbhak)

Samples	UV 365 nm		After Spraying/ Divertization	
	No. of spots	R _f Value	No. of spots	R _f Value
S1	9	0.12, 0.16, 0.20, 0.44, 0.50, 0.54, 0.66, 0.70, 0.85	6	0.12, 0.18, 0.29, 0.38, 0.52, 0.64
S2	6	0.18, 0.32, 0.46, 0.58, 0.64, 0.78	5	0.20, 0.34, 0.48, 0.56, 0.68
S3	7	0.12, 0.16, 0.22, 0.44, 0.54, 0.65, 0.72	6	0.12, 0.18, 0.28, 0.38, 0.54, 0.64

Study Photographs

1. Macroscopic Characters of *Jivak* & *Rishbhak*

Figure 1: *Jivak*-*Crepidium acuminatum* (D.Don).



Figure 2: Inflorescence of *Jivak*.



Figure 3: *Rishbhak*- *Malaxis muscifera* (Lindl.).

2. Genuine & Procured Sample of *Jivak*, *Rishbhak* For Organoleptic Study



Figure 4: Genuine Sample of *Jivak*.



Figure 5: Market Sample in the name of *Jivak-Rishbhak*.



Figure 6: Genuine Sample of *Rishbhak*.

3. Transverse section of Jivak, Rishbhak & Market Sample.

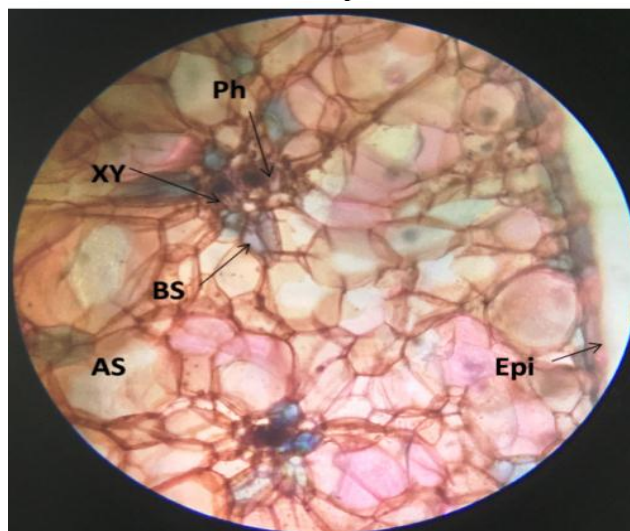


Figure 7: T.S Pseudobulb of *C.acuminatum* (D.Don).

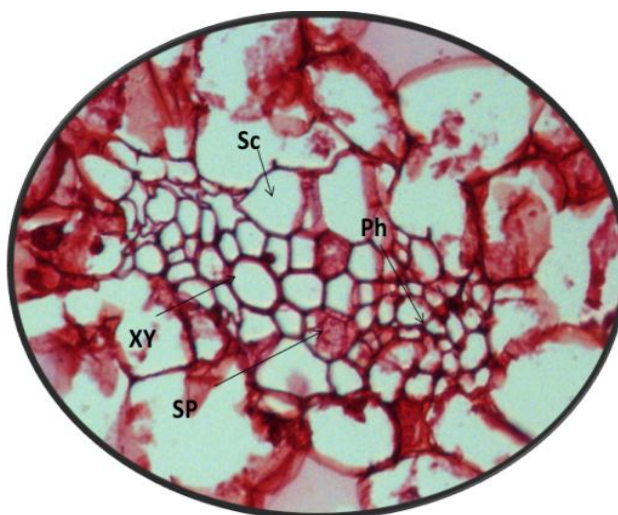


Figure 8: Vascular bundle of *C.acuminatum* (D.Don).

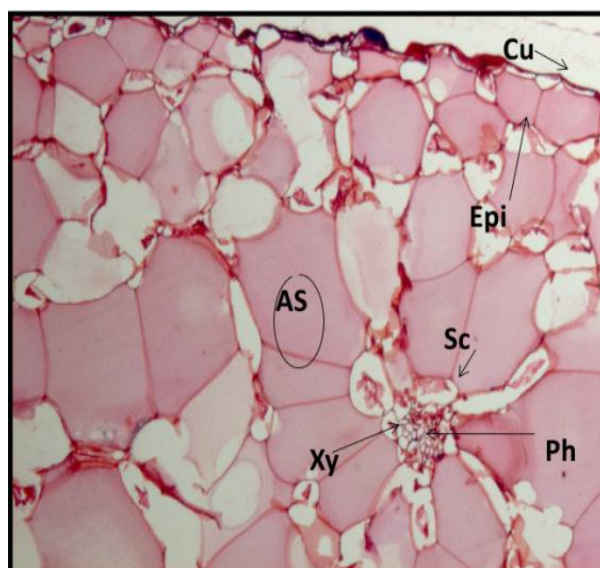
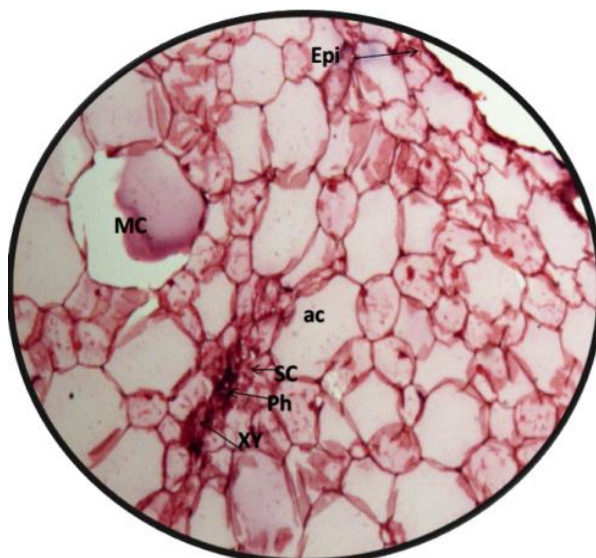


Figure 9: T.S of Market Sample under the name of *Jivak-Rishbhak*.

Figure 10: T.S of Pseudobulb *M.muscifera* (Lindl.).4. Thin Layer Chromatography of *Jivak*, *Rishbhak* & Market Sample– [Figure 11.]

Sample	<i>C.acuminatum</i> (<i>Jivak</i>)	<i>M.muscifera</i> (<i>Rishbhak</i>)	Market sample of <i>Jivak-Rishbhak</i>
TLC Plate			
R _f Value After Divertization	0.12, 0.18, 0.29, 0.38, 0.52, 0.64	0.20, 0.34, 0.48, 0.56, 0.68	0.12, 0.18, 0.28, 0.38, 0.54, 0.64

CONCLUSION AND RECOMMENDATIONS**CONCLUSION**

Finally it has been concluded that Pharmacognosy of medicinal herbs is very important as to confirm their identity, quality, purity, potency, safety and efficacy and in organoleptic, microscopic and T.L.C examination, procured market sample (S3) is found to be similar as *Jivak*. This means for commercial purpose may be somewhere tribal people are cultivating *Jivak*. But due to scarcity, *Rishbhak* is rarely available for medicinal purpose.

Future Recommendations

- ❖ Cultivation, collection, and preservation techniques for these plants should be developed.
- ❖ For the quality control, Biochemical biomarkers of these herbs should be investigated.
- ❖ Further work on Monograph of *Rishbhak* has to be done.
- ❖ The experimental and Clinical study should be conducted on these plants.
- ❖ Accurate knowledge about their medicinal uses, natural habitat, climate, soil adaptability, growing season, flowering time etc. should be generated.
- ❖ Drug standardization is also mandatory as it will provide reference standards for future. It will help in detection of adulteration or substitution and also maintain efficacy of these herbs.

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