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ANALYTICAL STUDY OF *SHANKHPUSHPI* & *MANDUKPARNI*

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ABSTRACT

Aim: *Shankhpushpi* & *Mandukparni* are to be taken for analytical study to match the standards of Ayurvedic Pharmacopoeia of India (API) with the aim to assess the safety and toxicity parameters of both the drugs, so that the drugs can be used in clinical study.

Methodology: The study is performed under following steps:

1. Collection of Plants
2. Powder Microscopy
3. Physicochemical Study
4. Phytochemical Study
5. Chromatography
6. Heavy Metal Analysis, Microbiological Study, Test for aflatoxins & Pesticide Residues

Observations & Results: The steps in methodology have been scientifically performed. Precise Observations thus obtained are noted and results have been prepared based upon the observations. All the parameters are matched with the given parameters in API. **Conclusion:** The analytical study has shown that the drugs are safe to be used in further experimental and clinical study as all parameters fall under the standard parameters of API.

INTRODUCTION

Shankhpushpi and *Mandukparni* are well described Ayurvedic herbs with their Ayurvedic properties and clinical uses. Both these drugs are classified as *Medhya* and indicated to be used in various mental and physical ailments while *Shankhpushpi* is specifically mentioned as *Medhya* among four *Medhya* drugs described by Acharya Charaka.[1] Analytical study of a medicinal plant includes pharmacognostical and phytochemical analysis. Pharmacognosy is the study of medicinal drugs derived from plants or other natural resources. The American society of Pharmacognosy defined Pharmacognosy as the study of physical, chemical, biochemical and biological properties of drugs, drug substances or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources.[2] It is also defined as the study of crude drugs. Phytochemicals are the various active chemical compounds found in plants. Phytochemicals (from Greek phyto, meaning "plant") are chemicals produced by plants through primary or secondary metabolism. They generally have biological activity in the plant host and play a role in plant growth or defense against competitors, pathogens, or predators.[3]

Shankhpushpi[4]

Latin Name : *Convolvulus pluricaulis syn. Convolvulus microphyllus*
Family : Convolvulaceae
Basonym : *Shankhpushpi*

Mandukparni[5]

Latin Name : *Centella asiatica*
Family : Apiaceae
Basonym : *Mandukparni*

METHODOLOGY: The study has been carried out under the following processes with their details -

1. Collection of Plants

Shankhpushpi- The original plants have been collected from the campus of *Gaur Brahman Ayurvedic College & Hospital, Rohtak, Haryana.* [Figure No. 1(a) & 1(b)] Herbarium was prepared for identification and authentification of the drug was done and the drug was found genuine. [Figure No. 2(a) & 2(b)].

Mandukparni- The original plants were grown and collected in the garden of *Rishikul Campus, Haridwar, Uttarakhand and Rajeshwari garden, Bahadarabad, Haridwar, Uttarakhand.* [Figure No. 3(a) & 3(b) respectively]. Herbarium was prepared for identification and authentification of the drug was done and the drug was found genuine. [Figure No. 4(a) & 4(b)].

2. Powder Microscopy: The collected sample of both plants were dried and Powder Microscopy was performed.

3. Physicochemical Study: Both the plants were evaluated in Laboratory for their Physicochemical parameters like Moisture content**,** pH**,** Alcohol Extractive Value**,** Aqueous Extractive Value**,** Petroleum Ether Extractive Value**,** Total Ash**,** Acid Insoluble Ash**,** Water Soluble Ash.

4. Phytochemical Study: Phytochemical analysis was done for raw drug and ghan-satva of both the plants. Raw drugs and freshly prepared extracts of both plants were tested for the presence of various active phytocompounds like Carbohydrates, Alkaloids, Amino acids, Proteins, Saponins, Glycosides, Phenolic compounds, Steroids, Tannins and Flavonoides.

5. Chromatography: Thin Layer Chromatography (TLC) was performed for raw drug and ghan satva of both plants and observations were recorded under UV Short wavelength and Iodine vapours.

6. Heavy Metal Analysis, Microbiological Study, Test for aflatoxins & Pesticide Residues: These parameters were evaluated for both the plant ghan satva and observations were recorded separately.

OBSERVATIONS AND RESULTS

A. *SHANKHPUSHPI*

1. Powder Microscopy [Figure No. 5]

S.No	Character	Chemical Reagent	Present/Absent
1.	Lignin (Fig. 5.1)	Phlorogucinol + HCl	Present
2.	Cellulose (Fig. 5.2)	Iodine Solution	Present
3.	Mucilage (Fig. 5.3)	Methylene Blue	Present
4.	Cell nuclei (Fig. 5.4)	Saffarinin	Present

2. Physico-Chemical Analysis

S.No.	Tests	*Shankhpushpi*	*Shankhpushpi Ghansatva*	API
1	Moisture content	6.63% v/w	20.82% v/w	-
2	Ph	5.2	5.1	-
3	Alcohol Extractive Value	18.23% w/w	25.37% w/w	> 6%
4	Aqueous Extractive Value	25.43 % w/w	100%w/w	> 10 %
5	Petroleum Ether Extractive Value	9.42% w/w	3.78 % w/w	-
6	Total Ash	8.68 % w/w	10.56 % w/w	< 17 %
7	Acid Insoluble Ash	2.95% w/w	3.58% w/w	< 8 %
8	Water Soluble Ash	5.56 % w/w	5.35% w/w	-

3. Phytochemical Analysis of Raw Drug

1. Carbohydrate test

Sr. no.	Name of test	Aqueous extract	Alcohol extract	Petroleum ether extract
A.	Molisch test	-ve	-ve	-ve
B.	Benedict test	+ve	+ve	-ve
C.	Barfoed's test	-ve	-ve	-ve
D.	Fehling test	+ve	+ve	-ve

2. Alkaloids

A.	Dragondrof test	+ve	+ve	-ve
B.	Mayer's test	-ve	-ve	+ve
C.	Wagner's test	-ve	-ve	-ve
D.	Hager's test	+ve	+ve	-ve

3. Amino acids

A.	Ninhydrine test	-ve	+ve	-ve

4. Proteins

A.	Biuret test	-ve	-ve	+ve
B.	Xanthoprotic test	-ve	-ve	+ve
C.	Millon's test	+ve	+ve	-ve

5. Saponin

A.	Foam test	+ve	-ve	-ve

6. Glycosides

A.	Borntragor's test	-ve	-ve	-ve

7. Phenolic compound

A.	Phenolic test	-ve	-ve	-ve

8. Steroids

A.	Salkowaski reaction	-ve	-ve	-ve

9. Tannin

A.	$Fecl_3$ test	-ve	-ve	-ve
B.	Lead acetate test	+ve	+ve	-ve
C.	Potassium dichromate test	+ve	+ve	-ve
D	Gelatin test	-ve	-ve	-ve

10. Flavonoids

A.	Shinods test	-ve	-ve	-ve

4. Phytochemical Analysis of *Shankhpushpi Ghansatva*

1. Carbohydrate test

Sr. no.	Name of test	Aqueous extract	Alcohol extract
A.	Molisch test	-ve	-ve
B.	Benedict test	+ve	+ve
C.	Barfoed's test	-ve	-ve
D.	Fehling test	+ve	+ve

2. Alkaloids

A.	Dragondrof test	+ve	+ve
B	Mayer's test	-ve	-ve
C.	Wagner's test	-ve	-ve
D.	Hager's test	+ve	+ve

3. Amino acids

A.	Ninhydrine test	-ve	+ve

4. Proteins

A.	Biuret test	-ve	-ve
B.	Xanthoprotic test	-ve	-ve
C.	Millon's test	+ve	+ve

5. Saponin

A.	Foam test	+ve	-ve

6. Glycosides

A.	Borntragor's test	-ve	-ve

7. Phenolic compound

A.	Phenolic test	-ve	-ve

8. Steroids

A.	Salkowaski reaction	-ve	-ve

9. Tannin

A.	$Fecl_3$ test	-ve	-ve
B.	Lead acetate test	+ve	+ve
C.	Potassium dichromate test	+ve	+ve
D	Gelatin test	-ve	-ve

10. Flavonoids

A.	Shinods test	-ve	-ve

5. Thin Layer Chromatography [Figure No. 6]

Sample	Iodine Vapour (Fig. 6.1)		UV Short wavelength (Fig. 6.2)	
	No. of spots	Rf value	No. of spots	Rf value
Shankhpushpi	7	0.23, 0.29, 0.36, 0.51, 0.73, 0.79, 0.82	5	0.23, 0.29, 0.36, 0.51, 0.73
Shankhpushpi ghansatva	7	0.23, 0.29, 0.36, 0.51, 0.73, 0.79, 0.82	5	0.23, 0.29, 0.36, 0.51, 0.73

6. Heavy Metal Analysis

S. No.	Heavy Metal	*Shankhpushpi Ghansatva*	Permissible Limit
1	Lead	Not Detected	**10ppm**
2	Arsenic	Not Detected	**3ppm**
3	Cadmium	Not Detected	**0.3ppm**
4	Mercury	Not Detected	**1ppm**

7. Microbiological Analysis

S.No	Analysis	*Shankhpushpi*	*Shankhpushpi Ghansatva*	Possible limit
1	Total Aerobic Microbial count	8475cfu/gm	46578 cfu/gm	100000 cfu/gm
2	Total Yeast & Mould count	274 cfu/gm	421 cfu/gm	1000 cfu/gm

8. Tests For Aflatoxins

S.No	Test for Aflatoxins	*Shankhpushpi*	*Shankhpushpi Ghansatva*	Possible limit
1	Aflatoxin B1	Absent	Absent	0.5ppm
2	Aflatoxin B2	Absent	Absent	0.1ppm
3	Aflatoxin G1	Absent	Absent	0.5ppm
4	Aflatoxin G2	Absent	Absent	0.1ppm

9. Pesticide Residue

Organophosphates	***Shankhpushpi Ghansatva***
O,O,O-Triethyle thiophosphate	Not Detected
Thionazin	Not Detected
Sulfotep	Not Detected
Phorate	Not Detected
Dimethoate	Not Detected
Disulfoton	Not Detected
Methyle parathion	Not Detected
Parathion	Not Detected
Famphur	Not Detected

Organochlorines	***Shankhpushpi Ghansatva***
Alpha-BHC	Not Detected
Beta-BHC	Not Detected
Gamma-BHC	Not Detected
Delta-BHC	Not Detected
Heptachlor	Not Detected
Aldrin	Not Detected
Heptachlor epoxide	Not Detected
Cis-Chlordane	Not Detected
Trans-Chlordane	Not Detected
pp-DDE	Not Detected
Dieldrin	Not Detected
Endrin	Not Detected
Endosulfan II	Not Detected
pp-DDD	Not Detected
Endrin Aldehyde	Not Detected
Endosulfan sulfate	Not Detected
pp-DDT	Not Detected
Endrin ketone	Not Detected
Methoxychlor	Not Detected

B. *MANDUKPARNI*

1. Powder Microscopy [Figure No. 7]

S.No	**Character**	**Chemical Reagent**	**Present/Absent**
1.	Tannin (Fig. 7.1)	Dilute Ferric Chloride	Present
2.	Mucilage (Fig. 7.2)	Methylene Blue	Present
3.	Cutin (Fig. 7.3)	Sudan III	Present
4.	Cell nuclei (Fig. 7.4)	Safranin	Present

2. Physico-Chemical Analysis

S.No.	**Tests**	***Mandukaparni***	***Mandukaparni Ghansatva***	**API**
1	Moisture content	7.32 %v/w	21.15% v/w	-
2	Ph	4.5	4.6	-
3	Alcohol Extractive Value	16.45 % w/w	26.69% w/w	> 9%
4	Aqueous Extractive Value	27.43 % w/w	49.58%w/w	> 20%
5	Petroleum Ether Extractive Value	8.76% w/w	2.56 % w/w	-
6	Total Ash	14.64 % w/w	15.43% w/w	< 17%
7	Acid Insoluble Ash	4.34 % w/w	5.89% w/w	< 5%
8	Water Soluble Ash	8.66 %w/w	9.95% w/w	-

3. Phytochemical Analysis of Raw Drug

1. Carbohydrate test

Sr. no.	Name of test	Aqueous extract	Alcohol extract	Petroleum ether extract
A.	Molisch test	-ve	-ve	-ve
B.	Benedict test	+ve	+ve	-ve
C.	Barfoed's test	-ve	-ve	-ve
D.	Fehling test	-ve	+ve	-ve

2. Alkaloids

A.	Dragondrof test	+ve	+ve	-ve
B.	Mayer's test	+ve	+ve	-ve
C.	Wagner's test	-ve	-ve	-ve
D.	Hager's test	-ve	-ve	-ve

3. Amino acids

A.	Ninhydrine test	-ve	-ve	+ve

4. Proteins

A.	Biuret test	-ve	-ve	-ve
B.	Xanthoprotic test	+ve	+ve	-ve
C.	Millon's test	-ve	-ve	-ve

5. Saponin

A.	Foam test	+ve	-ve	-ve

6. Glycosides

A.	Borntragor's test	-ve	-ve	+ve

7. Phenolic compound

A.	Phenolic test	-ve	-ve	-ve

8. Steroids

A.	Salkowaski reaction	-ve	-ve	-ve

9. Tannin

A.	$Fecl_3$ test	-ve	-ve	-ve
B.	Lead acetate test	+ve	+ve	-ve
C.	Potassium dichromate test	-ve	+ve	-ve
D	Gelatin test	-ve	-ve	-ve

10. Flavonoids

A.	Shinods test	-ve	-ve	-ve

4. Phytochemical Analysis of *Mandukparni Ghansatva*

1.Carbohydrate test

Sr. no.	Name of test	Aqueous extract	Alcohol extract
A.	Molisch test	-ve	-ve
B.	Benedict test	+ve	+ve
C.	Barfoed's test	-ve	-ve
D.	Fehling test	-ve	+ve

2. Alkaloids

A.	Dragondrof test	+ve	+ve
B.	Mayer's test	+ve	+ve
C.	Wagner's test	-ve	-ve
D.	Hager's test	-ve	-ve

3. Amino acids

A.	Ninhydrine test	-ve	-ve

4. Proteins

A.	Biuret test	-ve	-ve
B.	Xanthoprotic test	+ve	+ve
C.	Millon's test	-ve	-ve

5. Saponin

A.	Foam test	+ve	-ve

6. Glycosides

A.	Borntragor's test	-ve	-ve

7. Phenolic compound

A.	Phenolic test	-ve	-ve

8. Steroids

A.	Salkowaski reaction	-ve	-ve

9. Tannin

A.	$Fecl_3$ test	-ve	-ve
B.	Lead acetate test	+ve	+ve
C.	Potassium dichromate test	-ve	+ve
D	Gelatin test	-ve	-ve

10. Flavonoids

A.	Shinods test	-ve	-ve

5. Thin Layer Chromatography [Figure No. 8]

Sample	Iodine Vapour (Fig. 8.1)		UV Short wavelength (Fig. 8.2)	
	No. of spots	Rf value	No. of spots	Rf value
Mandukparni	4	0.36, 0.38, 0.41, 0.59	3	0.36, 0.41, 0.59
Mandukparni Ghansatva	4	0.36, 0.38, 0.41, 0.59	3	0.36, 0.41, 0.59

6. Heavy Metal Analysis

S. No.	Heavy Metal	*Mandukaparni Ghansatva*	Permissible Limit
1	Lead	Not Detected	**10ppm**
2	Arsenic	Not Detected	**3ppm**
3	Cadmium	Not Detected	**0.3ppm**
4	Mercury	Not Detected	**1ppm**

7. Microbiological Analysis

S.No	Analysis	*Mandukaparni*	*Mandukaparni Ghansatva*	Possible limit
1	Total Aerobic Microbial count	9485 cfu/gm	5635 cfu/gm	100000 cfu/gm
2	Total Yeast & Mould count	324 cfu/gm	413 cfu/gm	1000 cfu/gm

8. Tests for Aflatoxins

S.No	Test for Aflatoxins	*Mandukaparni*	*Mandukaparni Ghansatva*	Possible limit
1	Aflatoxin B1	Absent	Absent	0.5ppm
2	Aflatoxin B2	Absent	Absent	0.1ppm
3	Aflatoxin G1	Absent	Absent	0.5ppm
4	Aflatoxin G2	Absent	Absent	0.1ppm

9. Pesticide Residue Analysis

Organophosphates	Mandukaparni Ghansatva
O,O,O-Triethyle thiophosphate	Not Detected
Thionazin	Not Detected
Sulfotep	Not Detected
Phorate	Not Detected
Dimethoate	Not Detected
Disulfoton	Not Detected
Methyle parathion	Not Detected
Parathion	Not Detected
Famphur	Not Detected

Organochlorines	Mandukaparni Ghansatva
Alpha-BHC	Not Detected
Beta-BHC	Not Detected
Gamma-BHC	Not Detected
Delta-BHC	Not Detected
Heptachlor	Not Detected
Aldrin	Not Detected
Heptachlor epoxide	Not Detected
Cis-Chlordane	Not Detected
Trans-Chlordane	Not Detected
pp-DDE	Not Detected
Dieldrin	Not Detected
Endrin	Not Detected
Endosulfan II	Not Detected
pp-DDD	Not Detected
Endrin Aldehyde	Not Detected
Endosulfan sulfate	Not Detected
pp-DDT	Not Detected
Endrin ketone	Not Detected
Methoxychlor	Not Detected

Figure No. 1(a) &1(b) [Collection of *Shankhpushpi*]

Fig. 1(a)

Fig. 1(b)

Figure No. 2(a) 2(b)
[Herbarium and Authentication of *Shankhpushpi*]

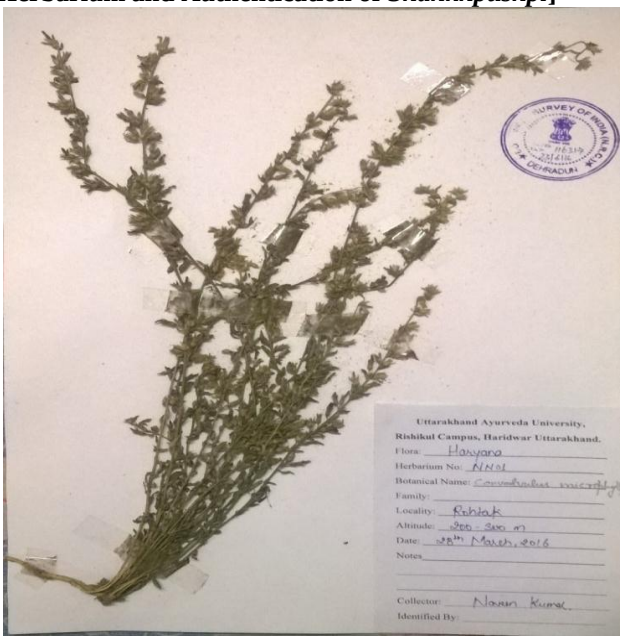
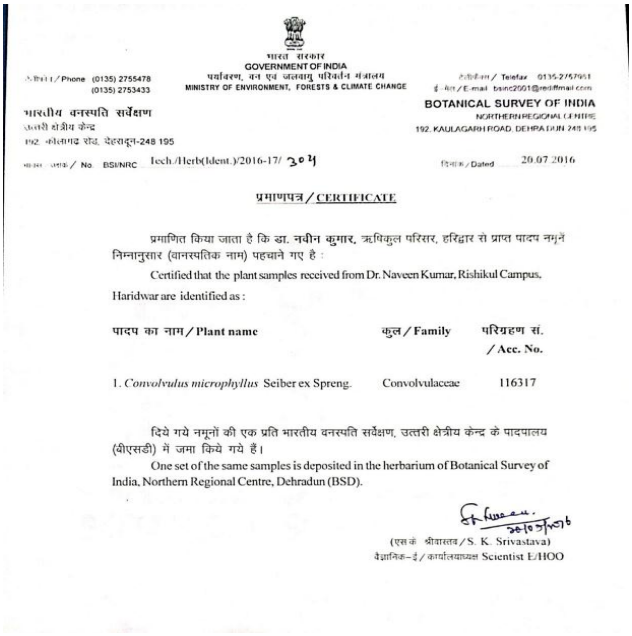


Fig. 2(a)



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192, कौलागढ़ रोड, देहरादून-248 195

No. BSI/NRC Tech./Herb(Ident.)/2016-17/ ३०५ Dated 20.07.2016

प्रमाणपत्र / CERTIFICATE

प्रमाणित किया जाता है कि डा. नवीन कुमार, ऋषिकुल परिसर, हरिद्वार से प्राप्त पादप नमूने निम्नानुसार (वानस्पतिक नाम) पहचाने गए है :

Certified that the plant samples received from Dr. Naveen Kumar, Rishikul Campus, Haridwar are identified as :

पादप का नाम / Plant name	कुल / Family	परिग्रहण सं. / Acc. No.
1. *Convolvulus microphyllus* Seiber ex Spreng.	Convolvulaceae	116317

दिये गये नमूनों की एक प्रति भारतीय वनस्पति सर्वेक्षण, उत्तरी क्षेत्रीय केन्द्र के पादपालय (बीएसडी) में जमा किये गये हैं।

One set of the same samples is deposited in the herbarium of Botanical Survey of India, Northern Regional Centre, Dehradun (BSD).

20/07/2016

(एस के श्रीवास्तव / S. K. Srivastava)
वैज्ञानिक-ई / कार्यालयाध्यक्ष Scientist E/HOO

Fig. 2(b)

Figure 3(a) & 3(b) [Collection of *Mandukparni*]

Fig. 3(a)

Fig. 3(b)

Figure No. 4(a) & 4(b)
[Herbarium and Certificate of Authentication of *Mandukparni*]

Fig. 4(a)

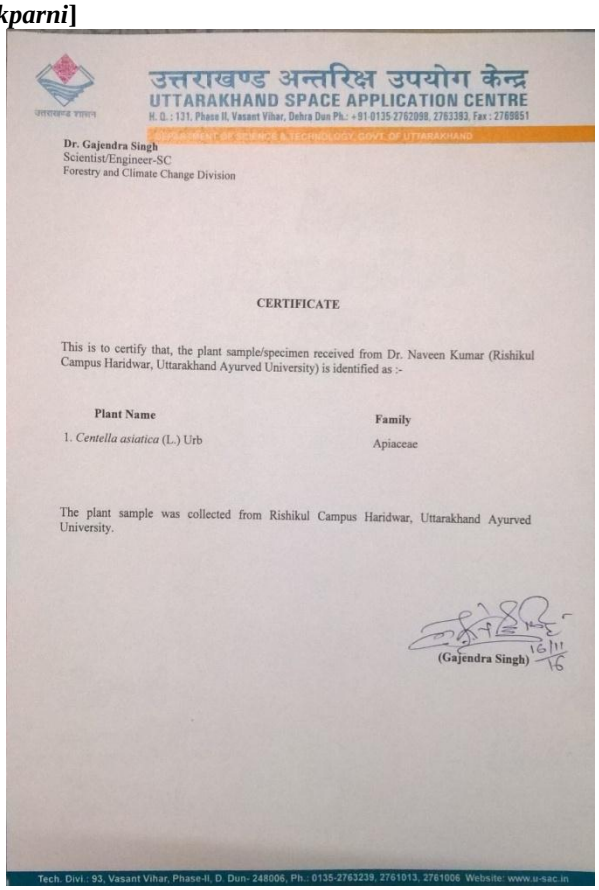


Fig. 4(b)

PLATE NO. 5 [Powder Microscopy of *Shankhpushpi*]

Fig. 5.1 Red Colour of Lignine (Stain: Phlorogucinol + HCl)

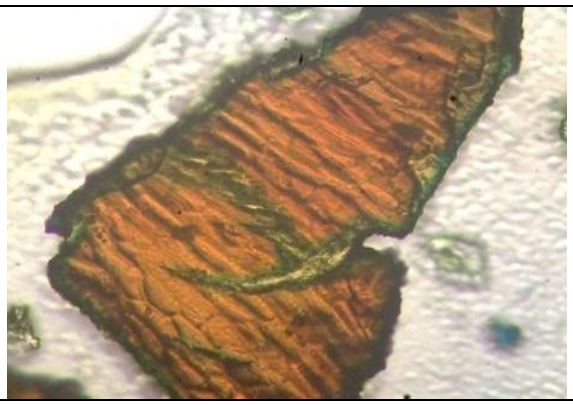


Fig. 5.2 Pale yellow Colour of Cellulose (Stain: Iodine Solution)

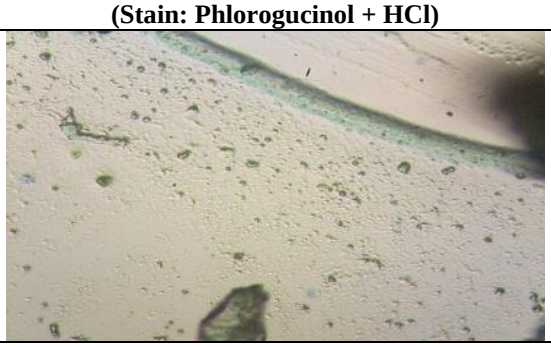


Fig. 5.3 Deep Blue Colour of Mucilage (Stain: Methylene Blue)

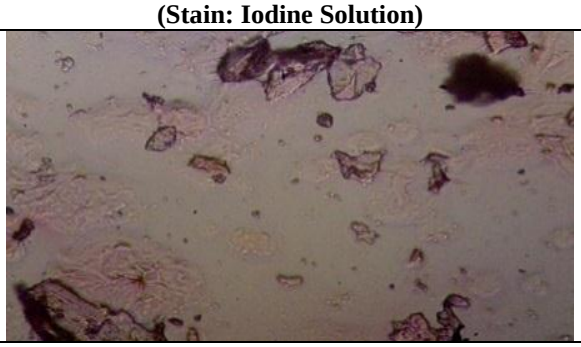


Fig. 5.4 Red Colour of cell nuclei (Stain: Safranin)

PLATE NO. 6 [Thin Layer Chromatography of *Shankhpushpi*]

6.1. In Iodine Vapour

Samples	*Shankhpushpi*	*Shankhpushpi Ghansatva*
TLC Plate		
R_f Value	0.23, 0.29, 0.36, 0.51, 0.73, 0.79, 0.82	0.23, 0.29, 0.36, 0.51, 0.73, 0.79, 0.82

6.2. In UV Short Wavelength

Samples	*Shankhpushpi*	*Shankhpushpi Ghansatva*
TLC Plate		
R_f Value	0.23, 0.29, 0.36, 0.51, 0.73	0.23, 0.29, 0.36, 0.51, 0.73

Figure No. 7 [Powder Microscopy of *Mandukparni*]

Fig. 7.1 Green colour of Tannins
(Stain: Dil. Ferric Chloride Solution)

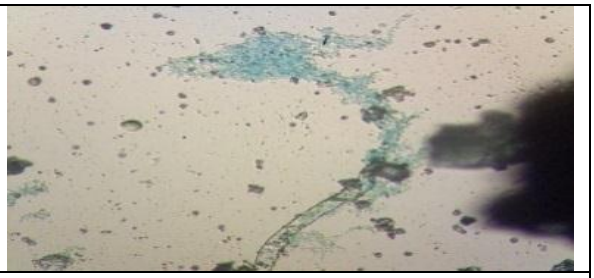


Fig. 7.2 Deep Blue Colour of Mucilage
(Stain: Methylene Blue)

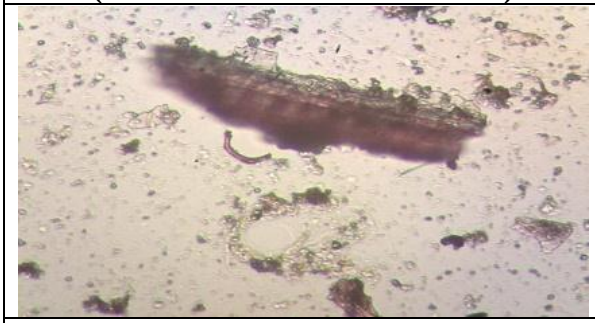


Fig. 7.3 Red Colour of Cutin
(Stain: Sudan III)

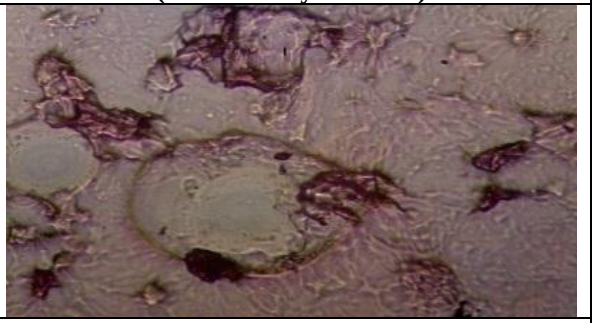


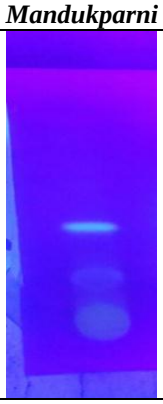
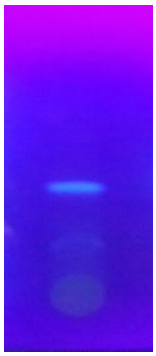
Fig. 7.4 Red Colour of cell nuclei
(Stain: Safranin)

PLATE NO. 8 [Thin Layer Chromatography of *Mandukparni*]

8.1. In Iodine Vapour

Samples	*Mandukparni*	*Mandukparnii Ghansatva*
TLC Plate		
R_f Value	0.36, 0.38, 0.41, 0.59	0.36, 0.38, 0.41, 0.59

8.2. In UV Short Wavelength

Samples	*Mandukparni*	*Mandukparnii Ghansatva*
TLC Plate		
R_f Value	0.36, 0.41, 0.59	0.36, 0.41, 0.59

CONCLUSION

Shankhpushpi- The raw drug collected from the natural habitat in fresh form and the species identified by the BSI, Dehradun. Thin layer chromatography has shown seven R_f values at 0.23, 0.29, 0.36, 0.51, 0.73, 0.79, 0.82 for *Shankhpushpi* Ghansatva when observed in Iodine vapours while shows four R_f Values 0.23, 0.29, 0.36, 0.51, 0.73 when observed in UV Short wavelength. Powder microscopy of *Shankhpushpi* has detected the presence of Lignine, Cellulose, Mucilage and red coloured cell nuclei. Heavy metal analysis confirmed the absence of heavy metals in the *Shankhpushpi* ghan satva. Pesticide residues were not detected in the *Shankhpushpi* ghan satva. Total aerobic microbial count & total yeast and mould count showed the presence of microbes under permissible limits of API. After analytical evaluations, the drug was found genuine and safe for the further use in experimental and clinical study.

Mandukparni- The drug was cultivated naturally and then species was identified by Uttarakhand Space Application Center. Thin layer chromatography for the *Mandukparni* ghansatva has shown four Rf values at 0.36, 0.38, 0.41, 0.59 when observed under Iodine Vapours while three Rf values at 0.36, 0.41, 0.59 when observed under UV short wavelength. Powder microscopy has confirmed the presence of Tannin, Mucilage, Cutin and cell nuclei. Heavy metal analysis confirmed the absence of heavy metals in the *Shankhpushpi* ghan satva. Pesticide residues were not detected in the *Mandukparni* ghan satva. Total aerobic microbial count & total yeast and mould count showed the presence of microbes under permissible limits of API. After analytical evaluations, the drug was found genuine and safe for the further use in experimental and clinical study.

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