ejpmr, 2020,7(10), 566-577

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

SJIF Impact Factor 6.222

Research Article ISSN 2394-3211 EJPMR

ANALYTICAL STUDY OF SHANKHPUSHPI & MANDUKPARNI

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Article Received on 10/08/2020	Article Revised on 31/08/2020	Article Accepted on 21/09/2020

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.



Tannins and Flavonoides.

Short wavelength and Iodine vapours.

observations were recorded separately.

Saponins, Glycosides, Phenolic compounds, Steroids,

5. Chromatography: Thin Layer Chromatography

(TLC) was performed for raw drug and ghan satva of

both plants and observations were recorded under UV

6. Heavy Metal Analysis, Microbiological Study, Test

for aflatoxins & Pesticide Residues: These parameters

were evaluated for both the plant ghan satva and

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,

OBSERVATIONS AND RESULTS A. *SHANKHPUSHPI* 1. Powder Microscopy [Figure No. 5]

y [rigui	Te 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
А.	Molisch test	-ve	-ve	-ve
В.	Benedict test	+ve	+ve	-ve
C.	Barfoed's test	-ve	-ve	-ve
D.	Fehling test	+ve	+ve	-ve

2. Alkaloids

А.	Dragondrof test	+ve	+ve	-ve
В.	Mayer's test	-ve	-ve	+ve
C.	Wagner's test	-ve	-ve	-ve
D.	Hager's test	+ve	+ve	-ve

3. Amino acids

А.	Ninhydrine test	-ve	+ve	-ve
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4. Proteins

А.	Biuret test	-ve	-ve	+ve
В.	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

 Phenolic compound

 A.
 Phenolic test
 -ve
 -ve
 -ve

8. Steroids

A. Salkowaski reaction -ve -ve -ve

9. Tannin

А.	Fecl ₃ test	-ve	-ve	-ve
В.	Lead acetate test	+ve	+ve	-ve
C.	Potassium	+ve	+ve	-ve
	dichromate test			
D	Gelatin test	-ve	-ve	-ve

10. Flavonoids

Α.	Shinods test	-ve	-ve	-ve
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4. Phytochemical Analysis of Shankhpushpi Ghansatva

1. Carbohydrate test

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

2. Alkaloids

Α.	Dragondrof test	+ve	+ve
В	Mayer's test	-ve	-ve
C.	Wagner's test	-ve	-ve
D.	Hager's test	+ve	+ve

3. Amino acids

Α.	Ninhydrine test	-ve	+ve
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4. Proteins

Α.	Biuret test	-ve	-ve
В.	Xanthoprotic test	-ve	-ve
C.	Millon's test	+ve	+ve

5. Thin Layer Chromatography [Figure No. 6]

Samula	Iodine	Vapour (Fig. 6.1)	UV Short wavelength (Fig. 6.2)	
Sample	No. of spots	Rf value	No. of spots	Rf value
Shankhnushni	7	0.23, 0.29, 0.36, 0.51,	5	0.23, 0.29, 0.36, 0.51,
Shanknipushpi		0.73, 0.79, 0.82		0.73
Shankhpushpi	7	0.23, 0.29, 0.36, 0.51,	5	0.23, 0.29, 0.36, 0.51,
ghansatva	/	0.73, 0.79, 0.82	3	0.73

6. Heavy Metal Analysis

S No Heavy		Shankhpushpi	Permissible
5. 140.	Metal	Ghansatva	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

5. Saponin



6. Glycosides

	А.	Borntragor's test	-ve	-ve
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7. Phenolic compound

	A	ι.	Phenolic test	-1	ve	-1	ve	
8. Steroids								
	A.	S	alkowaski reactio	n	-V	e	-ve	;

9. <u>Tannin</u>

А.	Fecl ₃ test	-ve	-ve
В.	Lead acetate test	+ve	+ve
C.	Potassium dichromate test	+ve	+ve
D	Gelatin test	-ve	-ve

10. Flavonoids

A. Shinods test -ve -ve

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 Chansatya
Almha DUC	Not Detected
Априа-БНС	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
А.	Molisch test	-ve	-ve	-ve
В.	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
В.	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

А.	Fecl ₃ test	-ve	-ve	-ve
В.	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
В.	Benedict test	+ve	+ve
C.	Barfoed's test	-ve	-ve
D.	Fehling test	-ve	+ve

2. Alkaloids

А.	Dragondrof test	+ve	+ve
В.	Mayer's test	+ve	+ve
C.	Wagner's test	-ve	-ve
D.	Hager's test	-ve	-ve

3. Amino acids

Α.	Ninhydrine test	-ve	-ve
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4. Proteins

А.	Biuret test	-ve	-ve
В.	Xanthoprotic test	+ve	+ve
C.	Millon's test	-ve	-ve

5. Saponin

A. Foam test +ve -ve

6. Glycosides

A. Borntragor's test -ve

7. Phenolic compound

8. Steroids

A. Phenolic test -ve -ve

-ve

-ve

-ve

A. Salkowaski reaction

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]

Sampla	Iodine V	Iodine Vapour (Fig. 8.1) UV Short wavelength		
Sample	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	9485	5635 cfu/gm	100000
1	Microbial count	cfu/gm		cfu/gm
2	Total Yeast &	324	413	1000
	Mould count	cfu/gm	cfu/gm	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

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	

9. Pesticide Residue Analysis

Organachlarinag	Mandukaparni	
Organocinormes	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. 2(a) 2(b) [Herbarium and Authentication of *Shankhpushpi*]



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



Fig. 1(a)



Fig. 1(b)

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Certi	वानस्पतिक नाम) पहचाने गए है :	मार, ऋषिकुल परिसर, हरिद्व	ार से प्राप्त पादप नमृनें
	fied that the plant samples received	from Dr. Naveen Kumar, R	ishikul Campus.
Haddana	11-11C-1		
Haridwar are	e identified as :		
पादप का ना	ाम / Plant name	कुल/Family	परिग्रहण सं.
			/ Acc. No.
1. Convolvu	lus microphyllus Seiber ex Spre	ng. Convolvulaceae	116317
दिये : (बीएसडी) में	गये नमूनों की एक प्रति भारतीय वन- जमा किये गये हैं।	स्पति सर्वेक्षण, उत्तरी क्षेत्रीय	केन्द्र के पादपालय
One	set of the same samples is deposit	ted in the herbarium of Bo	tanical Survey of
India, Northe	ern Regional Centre, Dehradun (E	3SD).	
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		(एस के शीवास्तव (2010 July
		tendos a combana	TH Scientist E/UOO
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	1. Convolva (হাই (থীংমর্গে) में One India, North	1. Convolvalus microphyllus Seiber ex Spre दिये गये नमूनों की एक प्रति भारतीय वन (वीएसडी) में जमा किये गये हैं। One set of the same samples is deposi India, Northern Regional Centre, Dehradun (I	 Convolvulus microphyllus Seiber ex Spreng. Convolvulaceae दिये गये नमूनों की एक प्रति भारतीय वनस्पति सर्वेक्षण, उत्तरी क्षेत्रीय (बीएसर्ज) में जमा किये गये हैं। One set of the same samples is deposited in the herbarium of Bo India, Northern Regional Centre, Debradun (BSD). (एस वं जीगासक / देशानिक-4/ कार्यालया)

Fig. 2(a)

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)

PLATE NO. 5 [Powder Microscopy of Shankhpushpi]



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

6.1. In Iodine Vapour

Samples	Shankhpushpi	Shankhpushpi Ghansatva
TLC Plate		Shanknpushpi Ghansaiva
P. Voluo		

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]



PLATE NO. 8 [Thin Layer Chromatography of *Mandukparni*] 8.1. In Iodine Vapour



8.2. In UV Short Wavelength



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 Rf 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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