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Pharmacognostical and Pharmaceutical Exploration of the traditional medicine *Ipomoea carnea* Jacq.

Research Article

Nilima Wadnerwar[1*], Meena Deogade[2], Monika Singh[3]

1. Associate Professor, Department of Agadtantra, Mahatma Gandhi Ayurved College, Hospital & Research Centre, Salod (H), Datta Meghe Institute of Higher Education and Research (Deemed to be University), Wardha, Maharashtra, India.
2. Associate Professor, Department of Dravyaguna, 3. SRF (Pharmacognosy), All India Institute of Ayurveda, New Delhi. India.

Abstract

Background: *Ipomoea carnea* Jacq is an ethnopharmacological drug and its leaves, roots and latex are used for various indications by traditional healers. But seeds are not used in the treatment as they are more toxic. As the herbal medicines are becoming endangered, ethnopharmacological drug can be used efficiently as they are available abundantly. But the toxicity should be reduced for safer use in the treatment. Aim: Seeds of *Ipomoea carnea* Jacq are studied in the present study to observe the macroscopic, microscopic, physicochemical and phytochemical changes in seeds after detoxification in different media. Methods and Materials: Detoxification method described in Ayurveda was used. *Ipomoea carnea* seeds were subjected to boiling for three hours with Cow's milk. The same procedure was followed for another sample separately with Cow urine, sour gruel, *Triphala (Emblica officinalis, Terminalia chebula* and *Terminalia bellirica*) decoction and Distilled water as media. One raw sample was kept for comparison. The transverse section of the raw seed was studied. Microscopy of powder, physicochemical and phytochemical analysis of all the six samples was conducted and the observations were compared among all the samples. Results: The toxic principle Swainsonine was estimated as 3% in all the samples at retention time 3 minutes. The numbers of peaks were reduced in all the treated samples except DWSIC in comparison with raw sample and 2-4 peaks are observed at retention time 1-3 minutes in all treated samples. A new phytoamine having concentration 12% was detected at retention time 17 minutes in all (crude and purified) samples. Conclusion: The morpho-anatomical characteristics of the seed remain same even after being treated with different liquid media. The changes observed in HPLC may be due to antagonist and antidotal action of media used for detoxification.

Key Words: *Ipomoea carnea,* Ethnopharmacology, *Shodhana,* Swainsonine.

Introduction

Traditional medicines have been used widely by Indian healers since long ago. Most of them are abundantly available which can fulfill the health needs. *Ipomoea carnea* Jacq from Convolvulaceae family is a locoweed. In Maharashtra, it is commonly known as *Besharam* meaning shameless due to its rampant spreading. It is used as folk medicine. (1) All the parts of the plant have medicinal properties. Its leaves are used for scorpion bite (2), fungal infection and as purgative.(3) The parts like roots, stem, and latex are used for various skin diseases. (3,4) Seeds and leaves are toxic for cattle. (5) They contain the Swainsonine as the toxic principle. Leaves and flowers of *Ipomoea carnea* contain Swainsonine, Calystegines B1, B2, B3 and C1 along with other phyto constituents.(6) But seeds contain only Swainsonine, Calystegines B1, B2, B3 and C1 and are not used as medicines because of its highly toxic nature. Swainsonine has antitumor activity in advanced malignancies.(7) Hence the seeds can be used for different cancer conditions.

Due to the deforestation and changing climatic conditions, a wide number of species of medicinal plants are getting endangered whereas in India, *Ipomoea carnea* grows naturally in waste lands, wet land, roadside, canals and drain banks abundantly. Such plants can be used as herbal medicines widely as an alternative to endangered species. Ayurveda has mentioned some detoxification or purification techniques to eliminate the unwanted effects or errors of the medicine. For the treatment, medicine or drug should be authentic. In pharmacognosy, transverse section and powder microscopy are used for the identification of drugs. Powder microscopy is an efficient method for the authentication of the drugs.(8,9) In the present study, an attempt has been made to use these techniques for identification of *Ipomoea carnea* and to observe the effect of *shodhana* on seeds of *Ipomoea carnea.* It may be helpful to check the purity

*** Corresponding Author:**
Nilima Wadnerwar
Associate Professor, Department of Agadtantra, Mahatma Gandhi Ayurved College, Hospital & Research Centre, Salod (H), Datta Meghe Institute of Higher Education and Research (Deemed to be University), Wardha, Maharashtra, India.
Email Id: dr.nilima_wadnerwar@rediffmail.com

of the drug or to check the adulterants present with the main drug. With the help of this principle described in Ayurveda, the ethnopharmacological, physicochemical and phytochemical properties of the drug were studied. This work will be helpful for further research on seed or other parts of the plant.

Methods

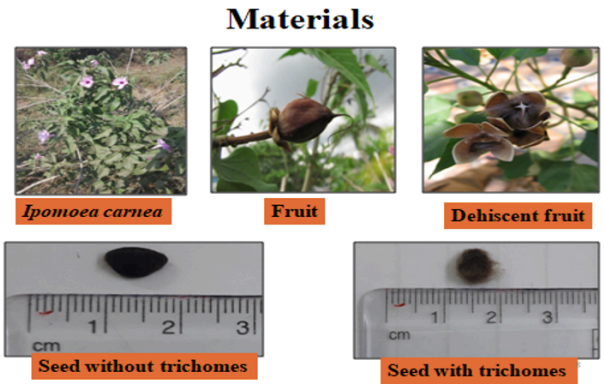
Experimental study was conducted after receiving IEC Approval Ref No. DMIMS (DU)/IEC/ Dec-2019/ 8629 dated: 21/12/2019.

Plant collection and identification

A field survey was conducted in the area surrounding Sawangi (Meghe), Salod (Hirapur), Deoli, Pulgaon, Paloti in Wardha district. Plant and seeds of *Ipomoea carnea* Jacq was identified and collected. It was authenticated from Foundation for Revitalization of Local Health Traditions, Bangalore. (FRLHT Col.No.123415 dated 29th May 2020).

The Good collection practices were undertaken for collection and preservation of the samples. Fully matured, self dried fruits of *Ipomoea carnea* were collected and seeds were separated from dried fruits. Physical impurities as well as trichomes (fur) attached with seeds were also removed. Fully matured black colored seeds were selected for the study. Weight, breadth and length of the fully matured single seed were recorded (Fig. 1).

Fig.1: Field sample of *Ipomoea Carnea* Jacq.



Detoxification /Purification of *Ipomoea carnea* seeds:

Mature seeds of *Ipomoea carnea* were divided in six groups. First sample was kept untreated. Second sample was treated by boiling with Cow milk for three hours. Third sample was treated with Cow urine. Fourth sample was treated with *Kanji* (sour gruel). Fifth sample was treated with *Triphala (Emblica officinalis, Terminalia chebula* and *Terminalia bellirica*) decoction and sixth sample was treated with distilled water with the help of method of boiling. All the groups were labelled as follows:

i. **ASIC-** Untreated seeds of *Ipomoea carnea*
ii. **GMSIC-** Seeds of *Ipomoea carnea* treated with Cow urine
iii. **KASIC-** Seeds of *Ipomoea carnea* treated with *Kanji* (sour gruel)
iv. **TKSIC-** Seeds of *Ipomoea carnea* treated with *Triphala* decoction
v. **GDSIC-** Seeds of *Ipomoea carnea* treated with Cow milk
vi. **DWSIC-** Seeds of *Ipomoea carnea* treated with Distilled water

Pharmacognostical study

It was conducted at Pharmacognosy Laboratory, All India Institute of Ayurveda, New Delhi. The plant material preserved in 10% FAA solution was used for microscopic study whereas for macroscopic and powder studies, the shade dried plant material was used.

Chemicals and reagents

All the chemicals used in the study were of analytical grade and were procured from E. Merck India Limited.

Macroscopic analysis

The macroscopic characters of the raw drug were studied. These included analysis of the dried seed for characters such as size, shape, colour, etc.

Microscopic analysis

The preserved samples were washed in running water and then samples were processed in the dehydration series followed by embedding series. These specimens were used to cut hand section. Permanent slides were prepared by double staining procedure using Safranin and fast green and mounted with DPX mountant. (10,11) These slides were then observed under the microscope and photomicrographs were taken. For the qualitative and quantitative analysis of the drug, microscopic studies were performed and Trinocular Zeiss Primostar 3 microscope was used to take the photographs.

The powder analysis was performed by preparing powder of the shade dried material. For powder preparation dried sample was finely powdered using mortar and pestle. Powder was then treated with different solvents and grouped into 7 categories and powder microscopy was performed using different reagents.(10) All the observations were recorded under microscope.

Analysis of *Ipomoea carnea* seeds

All the six samples were subjected to physicochemical analysis in which Moisture content, Total Ash Value, Water soluble Ash value, Acid insoluble ash value, Water extractive values, Alcohol extractive values, pH, Sieve analysis for particle size and Microbial examination was conducted. High Performance Liquid Chromatography (HPLC) was conducted.

HPLC (High Performance Liquid Chromatography):

Chemicals:

HPLC grade methanol and water were used for HPLC analysis. They were purchased from Merck (Mumbai MH. India). HPLC grade ammonium acetate

was purchased from Sigma-Aldrich (Mumbai MH, India). 0.2µ sample filters and 0.45µ nylon solvent filters were purchased from Milipore, India. Sample sonication was performed in Labman sonicator, purchased from Multilab, Ltd, Chennai, India.

Procedure

Exactly 700 mg of each sample was weighed and diluted with 1mL of IPA, 2 mL of water and 1mL of methanol. These samples were heated at 60^0 C for 5 minutes to assure complete dissolution of all components. Furthermore, they were sonicated and then filtered through 0.45uL nylon filters. 20 uL of each sample was then analyzed using HPLC instrument. Composition of methanol, water and ammonium acetate was used as mobile phase. Chromatographic analysis was performed with Shimadzu Prominence HPLC instrument. It is equipped with quaternary pump, (LC20 -80) degasser (DGU- 20As) column oven (CTO-10As) Auto sample (SIL-20 AC) Diode-Array-Detector (UVSPD-M20). Phytochemicals were analyzed with Prime SIL C_{18} column (250*4.6 mm. ID).

Results

Morphology

Ipomoea carnea is a 6 meters terrestrial plant. It bears a tap root having numerous lateral rootlets. Root is 50-60 cm in length and 2-3cm in diameter. Stem is thick which develops into a solid trunk over several years and it possesses many branches from its base. Stem is woody, erect, hairy, cylindrical in shape and green in colour.

Fruit

It is a simple fruit. It measures 2 cm in length and 1.5 cm in width. Immature fruit is green and mature fruit is brown in colour. It is non-fleshy and contains four black coloured seeds. Its dehiscence occurs when it becomes dry.

Seed

Macroscopic analysis

The seed of the *Ipomea carnea* is black in colour, elliptical in shape and measures 0.5-1 cm in length. It has wooly, silky brown trichomes. The seed is smooth to touch when the trichomes are removed.

Microscopic Analysis

Transverse section of the seed shows circular outline with outermost part having fibers, outermost layer is epicarp, toothed, single layered and pigmented. It is followed by a broad wavy mesocarp, cells parenchymatous, columnar and without intercellular spaces a few idioblastic cells containing starch grains, interrupted by canals. This is followed by a broad endocarp, cells parenchymatous, isodiametric, without intercellular spaces and a few idoblastic cells having rosette crystals of calcium oxalate and oil globules. (Fig.2)

Powder Microscopy

***Ipomea carnea* untreated/ *Ashodhita* (ASIC):** The seed shows the presence of parenchyma cells, stone cells, prismatic crystals of calcium, Simple and compound starch grains and fibers of parenchymatous cells. (Fig.3)

***Ipomea carnea* treated with Cow urine (GMSIC):** The seed shows the presence of Parenchyma cells, prismatic crystals of calcium, Simple and compound starch grains. (Fig.4)

***Ipomea carnea* treated with sour gruel *(Kanji)* (KASIC):** The seed shows the presence of Parenchyma cells, stone cells, prismatic crystals of calcium, Simple & compound starch grains. (Fig.5)

***Ipomea carnea* treated with *Triphala* decoction (TKSIC):** The seed shows the presence of parenchyma cells, prismatic crystals of calcium, Simple & compound starch grains. (Fig.6)

***Ipomea carnea* treated with Cow milk (GDSIC):** The seed shows the presence of parenchyma cells, prismatic crystals of calcium, simple & compound starch grains. (Fig.7)

***Ipomea carnea* treated with Distilled water (DWSIC):** The seed shows the presence of parenchyma cells, prismatic crystals of calcium, Simple & compound starch grains. (Fig.8)

Results

Table 1: Observations of physicochemical analysis

Sr. No.	Analytical Parameter	ASIC	GDSIC	GMSIC	KASIC	TKSIC	DWSIC
1	Moisture content at 105^0 C	0.54	0.72	0.41	0.68	0.51	0.32
2	Total Ash Value	5.33	6.27	6.27	6.82	5.97	4.29
	Water soluble Ash value	2.98	3.11	3.12	4.87	2.81	1.78
3	Acid insoluble Ash value	0.5	0.5	0.5	0.5	0.5	0.5
4	Water soluble extractive value	42.61	43.72	38.82	31.73	31.61	41.83
5	Alcohol soluble extractive value	22.71	29.12	26.88	26.90	41.72	24.27
6	pH	5.2	5.1	5.4	4.6	5.0	4.9
7	Particle Size (By Sieve Analysis using 80 No. Mesh)	100%	100%	100%	100%	100%	100%

Fig.2 *Ipomea carnea* Seed T.S.; A: At 10X showing, single layered epicarp, a broad mesocarp, parenchymatous endocarp, a few cells with starch; B: At 10X showing outermost fibers single layered epicarp, a broad mesocarp, a notch & parenchymatous endocarp Parenchyma cells; C: At 40X showing a broad parenchymatous endocarp; D: At 40X showing parenchymatous endocarp, rosette crystal of calcium oxalate & a few oil globules.

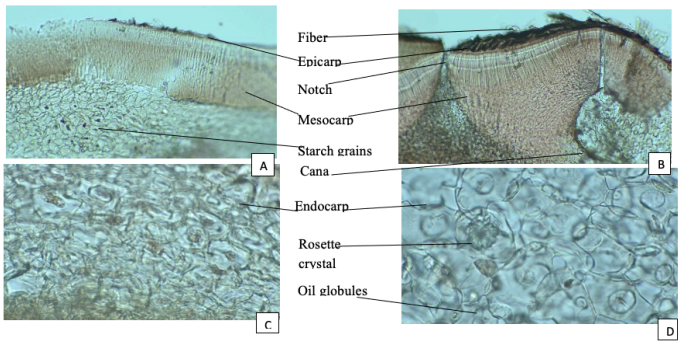


Fig.3 *Ipomea carnea* untreated (*Ashodhita*) (A-C) & covering (D); A: stained in safranin at 10X showing Parenchyma cells & stone cells; B: Stained in phloroglucinol at 10X showing parenchyma cells, prismatic crystals C: Stained in iodine at 40X showing Simple & compound starch grains D: Stained in safranin at 10X showing fibers of parenchymatous cells.

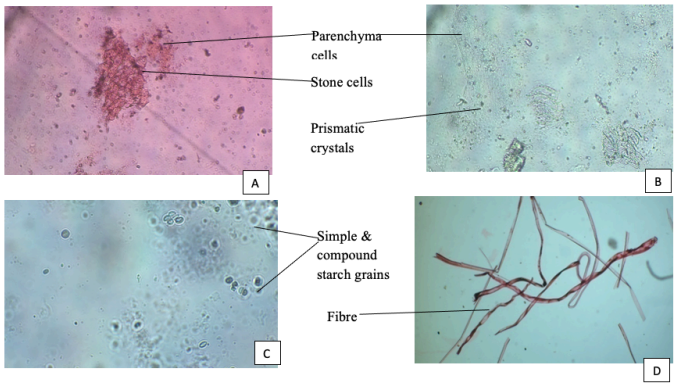


Fig.4 *Ipomea carnea* treated with Cow urine: A: stained in safranin at 10X showing Parenchyma cells; B: Stained in phloroglucinol at 10X showing Parenchyma cells, prismatic crystals C: Stained in iodine at 40X showing Simple & compound starch grains.

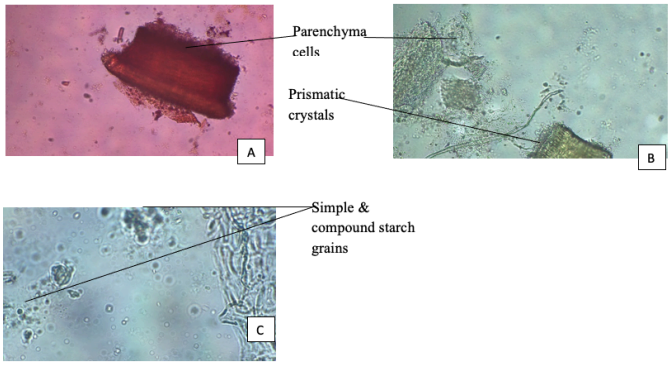


Fig.5 *Ipomea carnea* treated with sour gruel (*Kanji*): A: stained in safranin at 10X showing Parenchyma cells, stone cells; B: Stained in phloroglucinol at 10X showing, prismatic crystals C: Stained in iodine at 40X showing Simple & compound starch grains.

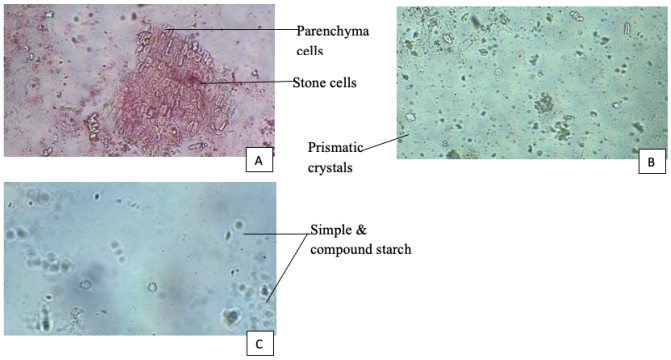


Fig.6 *Ipomea carnea* treated with *Triphala* decoction: A: stained in safranin at 10X showing Parenchyma cells; B: Stained in phloroglucinol at 10X showing prismatic crystals C: Stained in iodine at 40X showing Simple & compound starch grains.

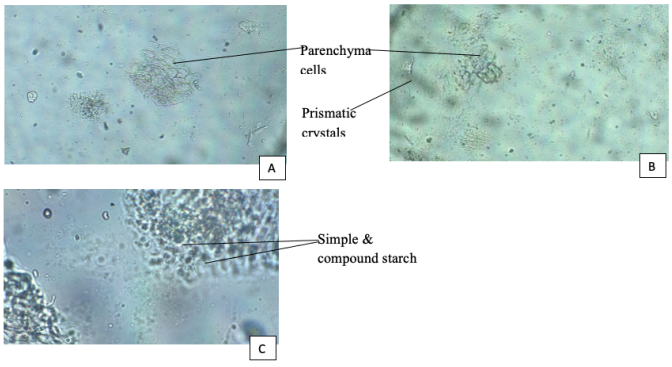


Fig.7 *Ipomea carnea* treated with Cow milk: A: stained in safranin at 10X showing Parenchyma cells; B: Stained in phloroglucinol at 10X showing parenchyma cells, prismatic crystals C: Stained in iodine at 40X showing Simple & compound starch grains.

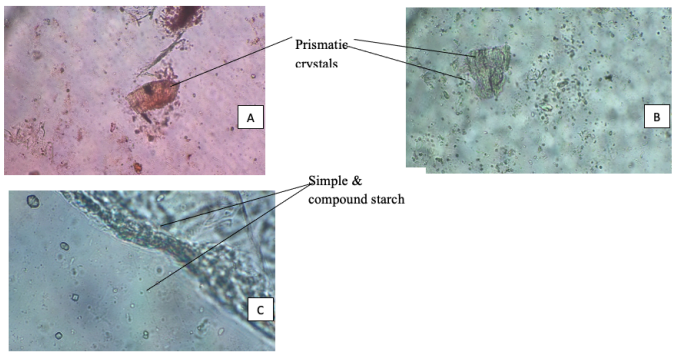
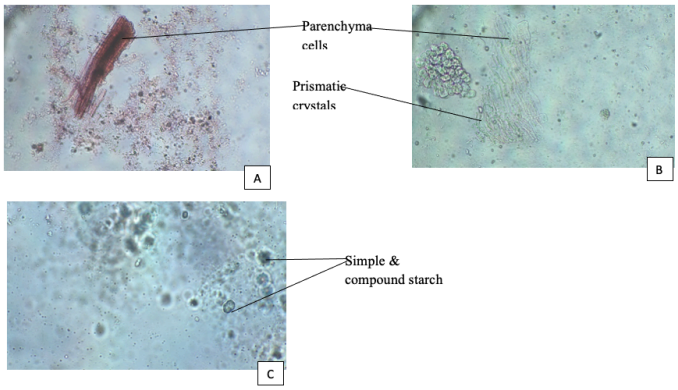


Fig.8 *Ipomea carnea* treated with Distilled water: A: stained in safranin at 10X showing Parenchyma cells; B: Stained in phloroglucinol at 10X showing parenchyma cells, prismatic crystals C: Stained in iodine at 40X showing Simple & compound starch grains.



Results of Phytochemical analysis

Fig.9 HPLC graph of ASIC (Detector A Ch2 254nm)

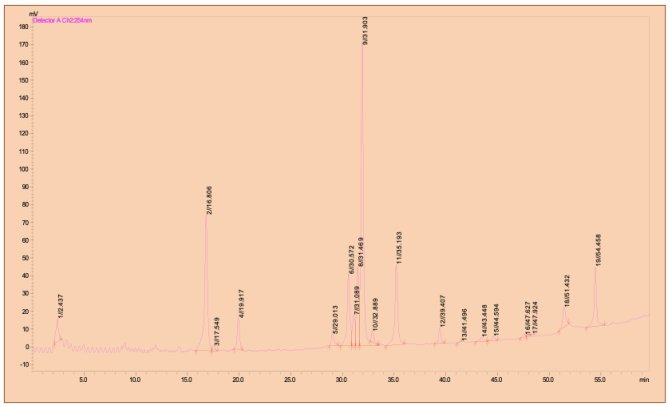


Fig.10 HPLC graph of GDSIC (Detector A Ch2 254nm)

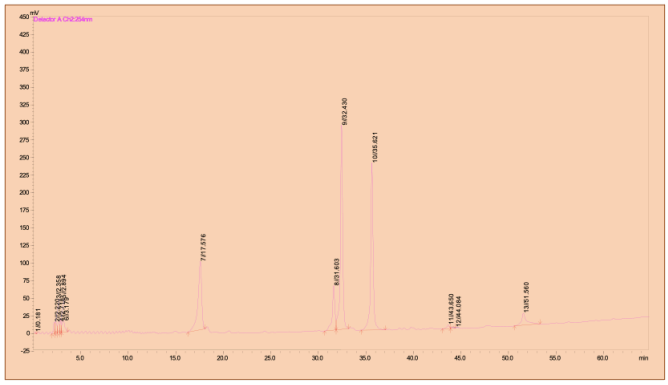


Fig.11 HPLC graph of GMSIC (Detector A Ch2 254nm)

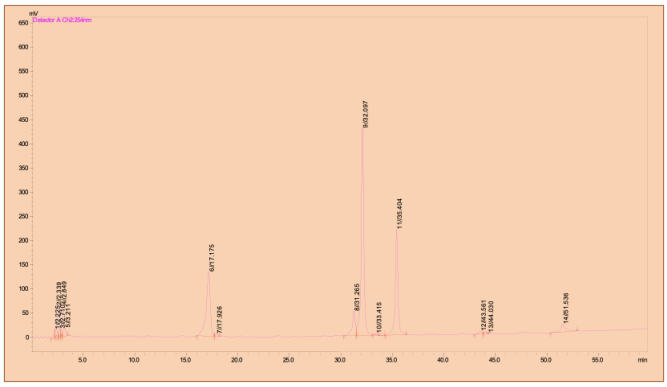


Fig.12 HPLC graph of KASIC (Detector A Ch2 254nm)

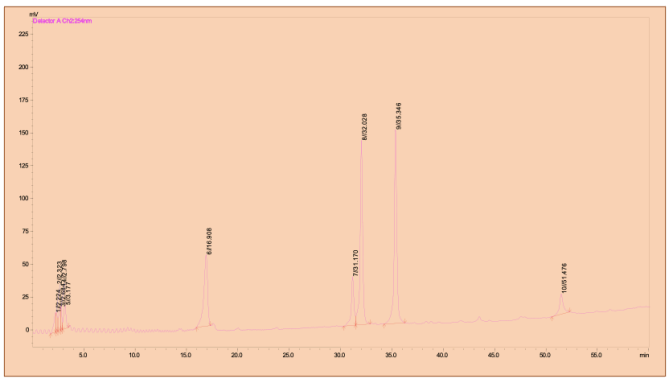


Fig.13 HPLC graph of TKSIC (Detector A Ch2 254nm)

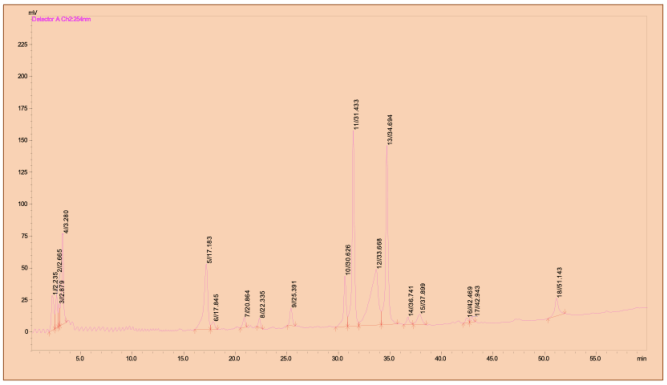
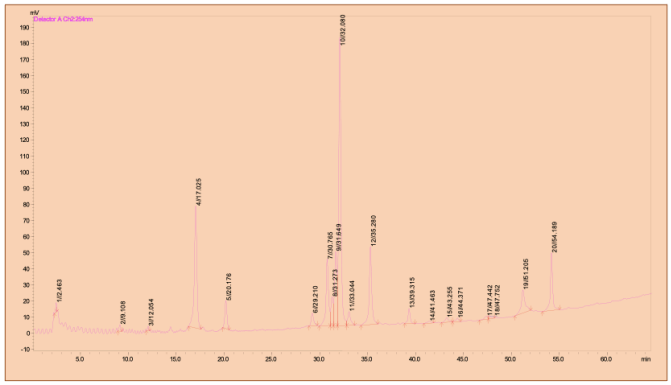


Fig.14 HPLC graph of DWSIC (Detector A Ch2 254nm



Discussion

Powder microscopy and pharmacognostical studies show that the morpho-anatomical characteristics of the seed remain same. They do not show any loss of anatomical features even after being treated with different liquid media. It clearly indicates that the method of *shodhana* do not interfere with the characters of the drug which are essential for its identification. It has been reported that the traditional medicines are being used since vedic times.(12) They can be utilized as a substitute for the species which are getting endangered.

In HPLC, the peak of Swainsonine was detected at retention time 3 minutes and it was estimated as 3% in all the samples. A new phytoamine has been detected at retention time 17 minutes in all (crude and purified) samples and it was estimated as 12%. The peaks of tannic acid and Gallic acid were found and estimated as 9% in all samples. The flavonoids viz Rutin Myricetin and Quercetin were detected as 65% in all samples. Quercetin is responsible for wound healing. 11 % unclassified terpenoids were detected only in untreated sample. Total 19 peaks were observed in untreated sample, 13 peaks were observed in GDSIC, 14 in GMSIC, 10 in KASIC, 18 in TKSIC and 20 in DWSIC at wavelength 254nm. Peaks from sample GDSIC, GMSIC, KASIC are decreased. It may be due to the antagonism action of treatment media over some poisonous compounds. 2-4 peaks are observed at retention time 1-3 minutes in GDSIC, GMSIC, KASIC

and TKSIC samples. It may be due to antidotal action of media used for treatment. The extinct components may be terpenoids which are not found in all treated samples. Hence, this treatment procedure may be helpful to reduce the harmful effect of *Ipomoea carnea* seeds.

A new phytoamine has been detected at retention time 17 minutes in all (crude and purified) extracts which was not characterized or reported in any other previous researches found on free accessed search engines. However, after comprehensive subject exploration, no any articles have displayed the HPLC report to understand the separation pattern and the location of constituents in *Ipomoea carnea* seed. Hence, there is a need to characterize the presence of the new phytoamine using more advanced tools like LC-MS/ MS.

Conclusion

Process of purification or detoxification do not affect the morphological characters which are essential for identification of the drug. Toxicity study of all the (crude and purified) samples of *Ipomoea carnea* seed is recommended in animals to evaluate the effect of *shodhana* on the toxic principle swainsonine and to confirm the toxic effects of untreated and treated samples of *Ipomoea carnea* seed. Further preclinical and cell line study is recommended to assess the efficacy of *Ipomoea carnea* seed in malignancy.

Conflict of Interest

None

Funding source

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