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Phytochemical and pharmacognostical evaluation of fresh and dry fruits of Shatapushpa (*Anethum sowa* Kurz)

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Abstract
The success of the treatment depends on the quality of each and every drug in a formulation. In Ayurvedic classics Acharyas have given prime importance to the drugs that are used for the preparation of medicines. In Sarangadhara Samhita, Rajanighantu Nighantu & Bhaishajyaratnavali it is mentioned that some drugs should be used in fresh form for preparing various Kalpanas [1-3]. Shatapushpa is one among them and is used in more than 30 classical as well as patent formulations. These preparations contain dry form of Anethum sowa KURZ. fruits instead of fresh. Thus, a study has been designed in order to compare the efficacy of Anethum sowa KURZ. fruits and to focus whether the drying has any influence in the phytoconstituents of fresh and dry forms. Commercially Shatapushpa fruits are dried under sunlight and hot air oven, in the present study we adopt three methods of drying. ie, drying under shade, sunlight and hot air oven.

Keywords: Fresh and dry fruits of Shatapushpa, pharmacognosy, phytochemical, HPTLC

Introduction
Anethum sowa KURZ is reputed as 'Indian dill' was used for culinary purpose from past decades in and around India. *Anethum sowa* KURZ is an annual flowering aromatic herb belongs to family Apiaceae, native to the Eastern Mediterranean region and West India. In India it was widely cultivated in tropical provinces of Madhya Pradesh, U. P, Punjab, Gujarath, Rajasthan etc. [4] Besides its culinary uses, the fruits were entrenched many Ayurvedic formulations. Pharmacological studies showed that the fruits have medicinal use as Diuretics, carminative, anti-bacterial, anti-septic, anti-spasmodic, digestant, bloodsugar, lowering, galactogogue & laxative [5]. Volatile principles of the fruit bestow its aromatic smell and virtuous flavour. Apiol is the foremost constituents of fruits of Indian dill [6].
The fruits of *Anethum sowa* KURZ was available in the market as dry form. Drying is one of the oldest and most extensively used methods of post-harvesting preservation [7]. Commercially *Anethum sowa* KURZ fruits were dried by different methods. So in the present study we adopt three methods of drying. i.e, drying under shade, sunlight and hot air oven. Sunlight drying was a widely used method. So it was included in the study As fruits of *Anethum sowa* KURZ contain more Volatile constituents [8], shade drying & sunlight drying were separately included under air drying methods. Hot air oven drying was a less time consuming drying method under controlled temperature. So it was included as one of the drying method. Thus, a study has been designed in order to compare whether the drying has any influence in the phytoconstituents of fresh and dry fruits of *Anethum sowa* KURZ by comparing the HPTLC profiling of each sample.

Materials and Methods
The proposed study was intended to compare the phytochemical elements of fresh & dried (in 3 different methods) fruits of Shatapushpa using HPTLC.
The study was conducted in 5 different steps;
1. Identification and Collection of fresh fruits of Shatapushpa.
2. Drying of fresh fruits in 3different methods.
3. Pharmacognostical, Physico-chemical analysis of fresh & dried fruits
4. phytochemical analysis of fresh& dried fruits
5. HPTLC

Identification & Method of collection of fresh fruits of *Anethum sowa* KURZ

The cultivated plant was identified by a taxonomist before the collection with the help of various vegetative and floral characters explained in different flora available [9, 10]. The matured fresh fruits of *Anethum sowa* KURZ were collected from the home garden, Kayamkulam, Alappuzha district, Kerala by hand picking in the month of November.

Drying of fruits

Drying of fresh fruits of *Anethum sowa KURZ* has been carried out by three different methods

- Under shade. (For 7 days)
- Under sunlight. (For 3 days)
- Under hot airoven. (For one and half hour at 60 degree Celsius)

Fresh and dried fruits of *Anethum sowa KURZ* were grouped as

- Sample 1- *Anethum sowa KURZ* fruit before drying
- Sample 2- *Anethum sowa KURZ* fruits after Shade drying
- Sample 3- *Anethum sowa KURZ* fruits after Sunlight drying
- Sample 4- *Anethum sowa KURZ* fruits after Hot air oven drying

Pharmacognostical and physico chemical analysis

Pharmacognostical analysis of fruits

Pharmacognostical study of fresh & dry fruits of Shatapushpa were carried out in Quality control lab, Amrita School of Ayurveda.

Macroscopic Evaluation [11]

Materials

Magnifying lens and dissecting microscope were used for this study.

Methodology of Macroscopic Evaluation

The collected samples of Shatapushpa were subjected to identification with naked eyes and by tactile and other sensory inspections. The macroscopic features of the collected sample drug were then compared with that of the description of *Anethum sowa KURZ* available in Ayurveda Pharmacopoeia of India and Quality standards of medicinal plants.

Microscopic Evaluation [12]

As Microscopic Features are same in fresh and dry fruits of shatapushpa as per API, only fresh fruit was taken for evaluating the histological parameters

Materials Required

Razor or safety razor blade, dissecting needles, watch glasses, microscope glass slides, cover slips, camel hair brush (medium size), dropper, glycerine, compound microscope.

After macroscopic evaluation, fine hand transverse section of fruit of *Anethum sowa KURZ* was made separately with the help of razor blade. The cut sections were then suspended in water in a watch glass. Then, it was transferred on a clean slide with help of a hair brush. The section was mounted at the centre of the slide and a drop of glycerine water was added on the section. Then it was covered with a cover slip without getting air bubble between the slide and cover. The prepared slide was placed on the stage of the compound microscope and fixed it with the clips. The light was focused to mounted slide by using the mirror. After this the lens was adjusted at a power of 10X for visualizing the histological parameters of the sample. Then the power was adjusted to 40X for getting finer details of the histological parameters. Photographs of the sections were taken using a digital camera at 10X and 40X powers. The histological features of the collected sample were then compared with that of the histological description of fruit of *Anethum sowa* KURZ available in Ayurveda Pharmacopoeia of India and other authentic text books.

Powder Microscopy [13]

The powder of fruit of *Anethum sowa* KURZ has been prepared in CARe KERALAM, Koratty.

Materials required

Fruits of Shatapushpa, Pestle & mortar, Sieve of mesh size 80.

Procedure

The collected fruits were dried and preserved was taken. Then it was powdered with the help of Pestle and Mortar and then it was sieved with a sieve of mesh size 80. A pinch of powder from this sample was taken on slide. This was spread well on the slide and the microscopic characters were observed after mounting in glycerine.

b. Physico chemical analysis of fruits [14]

Fresh & dry samples of Shatapushpa (*Anethum sowa* KURZ.) were subjected to physico chemical analysis in Quality Control Lab, CARe KERALAM, Koratty.

It includes;

1. Foreign matter
2. Loss on drying
3. pH value
4. Ash value
5. Acid insoluble ash
6. Water soluble extractive value
7. Alcohol soluble extractive value
8. Thin Layer Chromatography

Foreign matter

The 4 samples were spread on a thin layer in a wide tray separately and checked for any contaminations like moulds, insects, soil etc. with unaided eye or using a lens. The foreign matters were separated, weighed and total percentage was calculated

Loss on drying (L. O. D)

The instrument mains were switched on after ensuring it to be clean. After opening of the dome of the instrument an aluminium dish was placed on the tripod needle. The weight of the dish was tarred. About 2 g of the sample was evenly spread on the pan and the dome was closed. Heating was started automatically to evaporate the volatile and moisture from the sample. Reading was noted from the display monitor after the evaporation was over (after the beep sound of the machine).

pH value

5% solutions (2. 5 g) of 4 powdered samples were taken in 50 ml water seperately. Solutions were thoroughly mixed, kept aside for few minutes. It was stirred again and taken to check pH. The instrument was adjusted to pH mode. The electrode was washed with distilled water and wiped. The electrode was inserted into solutions and checked the pH. The values were noted. The values obtained were taken as the pH of the given

concentrated solutions. This process is continued for the remaining 3 samples.

Determination of Total ash

Accurately weighed 2 gms of powder of fresh and dry of fruits of Shatapushpa (*Anethum sowa* KURZ.) were taken in a previously ignited, cooled, weighed silica crucible. The samples were incinerated in an electric Bunsen burner by gradually increasing the heat not exceeding the dull red heat, cooled and weighed. Since a carbon free ash could not be obtained, the ashes were washed with water and collected the residue in Whatman No. 1 ashless filter paper; dried the filter paper containing the residue of the drug and ignited in a previously weighed silica crucible at a temperature not exceeding 450 degree Celsius till a constant weight was obtained. The percentage of total ash with respect to drugs was calculated.

$$\text{Percentage of Total ash} = \frac{(\text{wt of crucible + ash}) - \text{wt of crucible}}{\text{Wt of sample}} X\ 100$$

Determination of Acid insoluble ash

Accurately weighed 2 gms of powder of fresh & dry fruits of Shatapushpa (*Anethum sowa* KURZ.) were taken in previously ignited, cooled, weighed silica crucible. Ashes were obtained in the above same procedure and weighed. It was washed out in to 100ml beakers with 25 ml 2 N HCl and boiled for five minutes. The insoluble matter was collected on a Whatman No. 1 ash less filter paper, it was washed with hot distilled water for several times until free from chloride, dried and ignited in a previously weighed silica crucible at a temperature not exceeding 450 degree Celsius to get a constant weight. The percentage of acid insoluble ash in the samples were calculated with reference to the fresh and dried drug.

$$\%\ \text{of Acid insoluble ash} = \frac{(\text{wt of crucible +acid insoluble ash}) - \text{wt of crucible}}{\text{Wt of sample}} X\ 100$$

Determination of Water soluble ash

Ashes were prepared as per above procedure for 2 gm of fresh & dry powders of fruits of Shatapushpa (*Anethum sowa* KURZ.) It was completely transferred to a 100ml beaker by repeatedly washing with 25ml distilled water and boiled for five minutes. The insoluble matter was collected on ash less Whatman No. 1 filter paper, the residue on filter paper was washed with warm distilled water dried and ignited in a previously weighed dry silica crucible at a temperature not exceeding 450 degree Celsius till to get a constant weight. The percentage of water insoluble ash was calculated with reference to the fresh & dried drug.

$$\%\ \text{Water insoluble ash} = \frac{(\text{wt of crucible +water insoluble ash}) - \text{wt of crucible}}{\text{Wt of sample}} X\ 100$$

Determination of Extractive values

A. Alcohol soluble extractives

Mainly represent the percentage of organic plant constituents such as alkaloids, phenols, flavanoids, sugars, volatile oils, resins, steroids, glycosides etc. present in the drug.

Procedure

5 gm of accurately weighed fresh and dried powdered fruits of Shatapushpa (*Anethum sowa* KURZ.) were taken in 4 round bottom flasks. 100ml of 99% alcohol was added to each flask and were closed. The contents were occasionally shaken for first 6 hrs and then allowed to stand for next 18 hrs and this was rapidly filtered separately taking precautions against the loss of solvent. The filtrate was evaporated to dryness in two separate pre weighed beaker and dried at 100 degree Celsius. It was again weighed after heating and weighing continued till a constant weight was obtained. The percentage of cold alcohol soluble extractive was calculated with respect to fresh & dried drug.

$$\text{Percentage of Cold alcohol soluble extract} = \frac{\text{Wt. of cold alcohol soluble extract}}{\text{Wt. of drug}} x\ 100$$

= (Wt. of beaker + cold alcohol soluble extract) – (Wt. of beaker) / Wt. of drug x 100.

B. Water soluble extractives

Procedure

5 gm of accurately weighed coarsely powdered fresh & dried fruits of Shatapushpa (*Anethum sowa* KURZ) were transferred into 4 round bottom flasks. About 100ml of chloroform water (100ml distilled water + 2. 5 ml chloroform) was added to each flask and the contents were occasionally shaken for 24 hours. It was then filtered through filter paper and the filtrate obtained was evaporated to dryness at 110 degree Celsius in previously weighed beakers. Heating and weighing continued till a constant weight was obtained. The percentage of cold water soluble extractive was calculated with respect to the fresh & dried drugs were taken.

Percentage of Cold-water soluble extract = Wt. of cold-water soluble extract/ Wt. of drug x 100

Wt. of drug = (Wt. of beaker + cold water-soluble extract) – Wt. of beaker / Wt. of drug x 100

Phytochemical Analysis [15]

All the 4 samples were subjected to phytochemical analysis in Quality Control Lab, CARe KERALAM, Koratty.

Dragendorff's test for alkaloids

To a few ml of filtrate of four samples, 1 – 2 ml of Dragendorff's reagent were added. A prominent yellow precipitate was obtained in all 4 samples.

Shinoda's test for flavonoids: About 0. 5 of each portion was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips was then added to the filtrate followed by few drops of conc. HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids. The four samples did not show any coloured precipitate.

- **Benedict's test:** To 0. 5 ml of 4 filtrate, 0. 5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 min. A characteristic colored precipitate indicated the presence of sugar. The four samples did not show any precipitate
- **Test for Tannins:** About 0. 5 g each portion of four alcoholic extracts of samples were stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate. Occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins.

Sample 2 & 4 were found to be black in colour. Sample 1 & 3 did not have any colour change.

- **Detection of Saponins by Foam Test:** The four extracts (50 mg) were diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. A two cm layer of foam indicated the presence of saponins. In all the 4 samples layer of foams did not found.
- **Baljet test/picric acid test:** Reagent used in this test is picric acid and sodium picrate. Add the reagent to four extracts separately. Orange color is produced in the presence of cardiac glycoside. Orange colour obtained in all the 4 samples

High Performance Thin Layer Chromatography (HPTLC) [16]

All the 4 samples were subjected to HPTLC in CaRE Keralam, Koratty

Spotting of HPTLC plate: The prepared 4 methanolic extracts of the samples were applied in a dose of 20. 0µl at a position of 12. 5mm, 27. 5mm, 42. 5mm, 57. 5mm in HPTLC silica gel plate by Hamilton syringe at a speed of 150nl/s in CAMAG Linomat 5.

Preparation of TLC chamber: A clean beaker was taken and the mobile phase-Toluene: Chloroform: Methanol (8:3:1) poured into the beaker to height of 0. 5 to 0. 7 c. m and a filter paper of the length of the beaker was inserted. Mouth of the beaker was closed with a watch glass. The TLC plate was said to be saturated with the mobile phase when the solvent reaches the top of the filter paper.

Developing TLC: The spotted plate was slowly inserted into the TLC chamber opposite to the filter paper. The TLC plate was removed from the chamber when the solvent reaches almost to the top of the TLC plate. The point to which the solvent has reached TLC plate was marked a pencil. The plate was dried and subjected to visualization.

Visualization: Visualization of the developed TLC plate was done under visible light and UV long (366 nm). The plate was then exposed with derivatizing agents- iodine vapours and then sprayed with vanillin – sulphuric acid and heated for 10 minutes at 105 °C in hot air oven.

Rf value Calculation: Measured the distance of each spot from the points of its application and calculated Rf values by dividing the distance travelled by the spots by the distance travelled by the solvent front of the mobile phase.

Chromatogram evaluation: The wave length distribution of all spots of 4 samples obtained in TLC was monitored using software win CATS planar chromatography.

Observations and Results

Observations

Observation during drying

- After drying, the fragrance of the fruits increased.
- Colour of the fruits changed to grey.

Results of Pharmacognostic study

The macroscopic features, microscopy of the section and powder microscopy of the sample comply with API and Quality standards of Indian Medicinal Plants.

Macroscopy of fresh fruit of Shatapushpa

Colour: Greenish grey, stalk attached
Shape: Broadly oval and compress dorsally
Size: 4mm long, 2-3mm broad and 1mm thick
Texture: Mericarp separate and free traversed from the base to apex by 5 lighter coloured primary ridges of which 3 dorsal, slightly raised, brown, filiform and inconspicuous, 2 lateral prolonged into thin, yellowish membranous wings
Smell: Faintly aromatic
Taste: Pungent.

Microscopy of fresh fruit of Shatapushpa

Transverse section of fruit of Shatapushpa showed an outermost layer of pericarp, followed by mesocarp and an inner endocarp and endosperm & vascular bundles in winged costae.

Description of transverse section (T. S) of fresh fruit of *Anethum sowa* KURZ.

- Pericarp shows epidermis of polygonal tubular cells having thick outer wall and striated cuticle,
- Mesocarp, parenchymatous, some cells lignified and show reticulate thickening,
- Endocarp consists of tabular cells, sometimes with sinuous anticlinal walls, vittae, 4 on the dorsal valleculae and 2 on the commissural surface, extending the length of each mericarp with an endothelium of brown cells and containing volatile oil, dorsal costae3, one layer and the 2 lateral broadly winged each costae with vascular strands
- Endosperm much flattened and consist of thick-walled, cellulosic, parenchyma containing fixed oil and numerous aleurone grains up to 5µ in diameter
- containing micro rosette crystals of calcium,
- Carpophore split pairing at the apex into the raphe of each mericarp containing a vascular strand of sclerenchymatous fibres and spiral vessels.

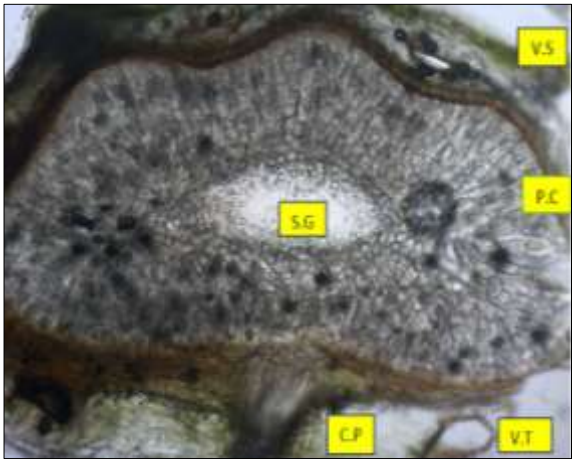

Fig 1: T. S of fruits of Shatapushpa, P. C-Parenchymatous cells, V. T-Vittae, C. P-Carpophore, V. S-Vascular Strands, S. G-Starch grains

Fig 2: Parenchymatous tabular cells

Fig 4: Carpophore

Fig 3: Vittae

Fig 5: Vascular strands

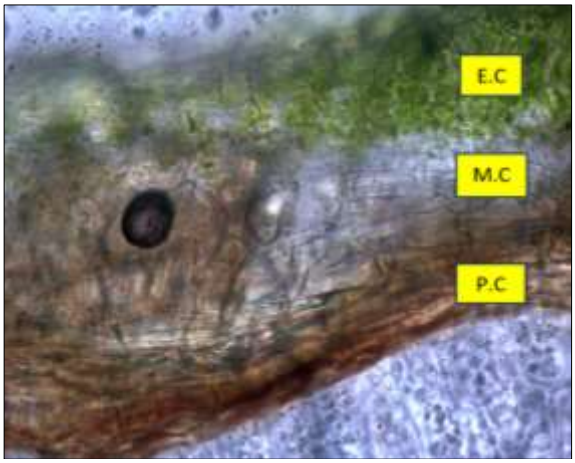

Fig 6: P. C-Pericarp, M. C-Mesocarp, E. C-Endocarp

Powder Microscopy

Powder Microscopy of fruit of *Anethum sowa* shows

- Brown in colour
- Spiral vessels
- Micro-rosette crystals of calcium oxalate, oil globules and aleurone grains.

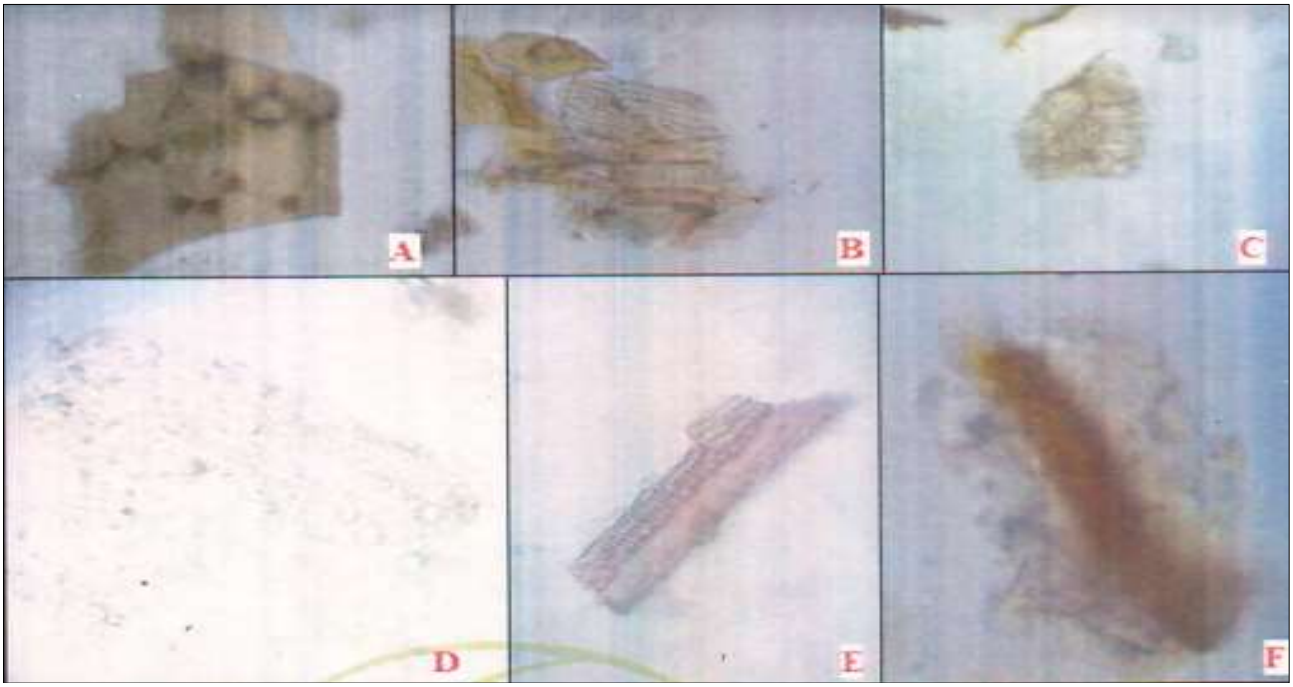

Fig 7: Powder microscopy of *Anethum sowa* KURZ A: Mesocarp cell with thick wall, B: Endocarp cell with surface view, C:Epicarp in surface view, D:Endosperm cells with Oil globules, E:Vascular elements, F:Fragments of vittae in surface view

Result of Physico chemical analysis of fruits of Shatapushpa before and after drying

Table 1: Results of physico chemical analysis of 4 samples of Shatapushpa.

SL. No	Parameters	Sample-1	Sample-2	Sample-3	Sample-4
1.	Foreign matter	-	-	-	-
2.	LOD	8. 84%	9. 29%	4. 83%	6. 91%
3.	pH	6. 77	6. 65	6. 66	6. 57
4.	Total ash	5. 68%	7. 39%	7. 25%	6. 98%
5.	Acid Insoluble ash	0. 33%	0. 01%	0. 08%	0. 06%
6.	Water extractives	17. 07%	14. 40%	18. 14%	19. 53%
7.	Alcohol extractives	5. 52%	6. 44%	7. 46%	7. 19%

Result of phytochemical analysis of fruits of shatapushpa before and after drying

Table 2: Result of phytochemical analysis of 4 samples of Shatapushpa.

SL No.	Constituents	Sample-1	Sample-2	Sample-3	Sample-4
1.	Alkaloids	Present	Present	Present	Present
2.	Flavonoids	Absent	Absent	Absent	Absent
3.	Phenol	Present	Present	Present	Present
4.	Saponins	Absent	Absent	Absent	Absent
5.	Tannins	Absent	Present	Absent	Present
6	Glycosides	Present	Present	Present	Present
7.	Carbohydrate	Absent	Absent	Absent	Absent

HPTLC of fresh and dry fruits of Shatapushpa

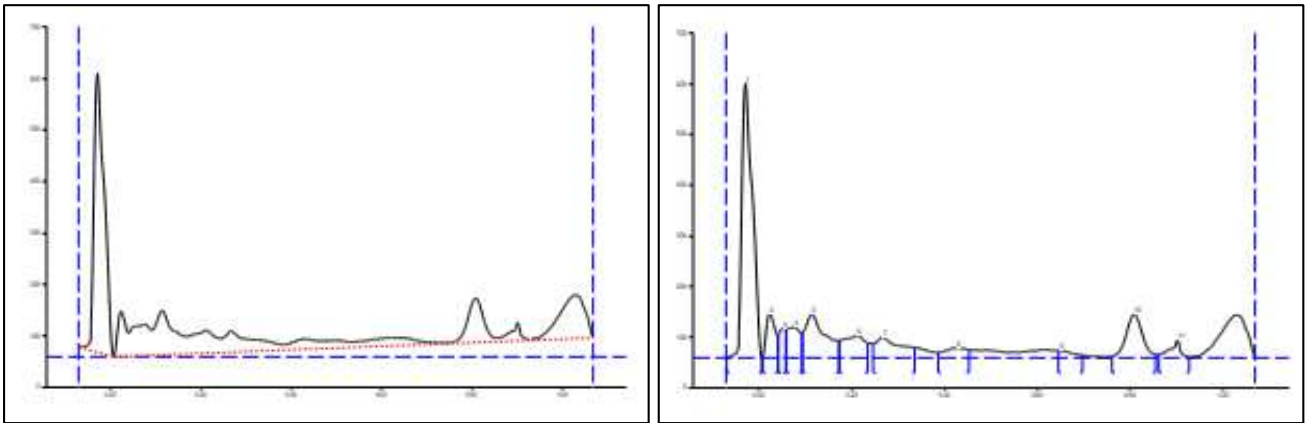

Fig 8: Wave length of compounds of Sample-1 at 366mm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.07	0.3	-0.03	541.9	50.21	0.01	0.1	9910.1	40.92
2	0.01	3.4	0.02	88.0	8.15	0.04	46.0	1340.7	5.54
3	0.04	47.2	0.05	58.7	5.43	0.06	57.9	724.3	2.99
4	0.06	57.6	0.08	61.2	5.67	0.09	49.5	1370.8	5.66
5	0.09	50.2	0.11	86.0	7.97	0.17	34.5	3057.5	12.63
6	0.17	34.9	0.21	44.8	4.15	0.23	30.2	1655.9	6.84
7	0.25	28.1	0.27	41.8	3.87	0.34	20.9	1835.0	7.58
8	0.39	10.9	0.43	20.9	1.94	0.45	16.5	810.2	3.35
9	0.65	14.5	0.65	15.2	1.41	0.70	5.3	402.3	1.66
10	0.76	3.1	0.81	86.1	7.97	0.86	7.7	2465.9	10.18
11	0.86	7.3	0.90	34.9	3.24	0.93	1.4	644.7	2.66

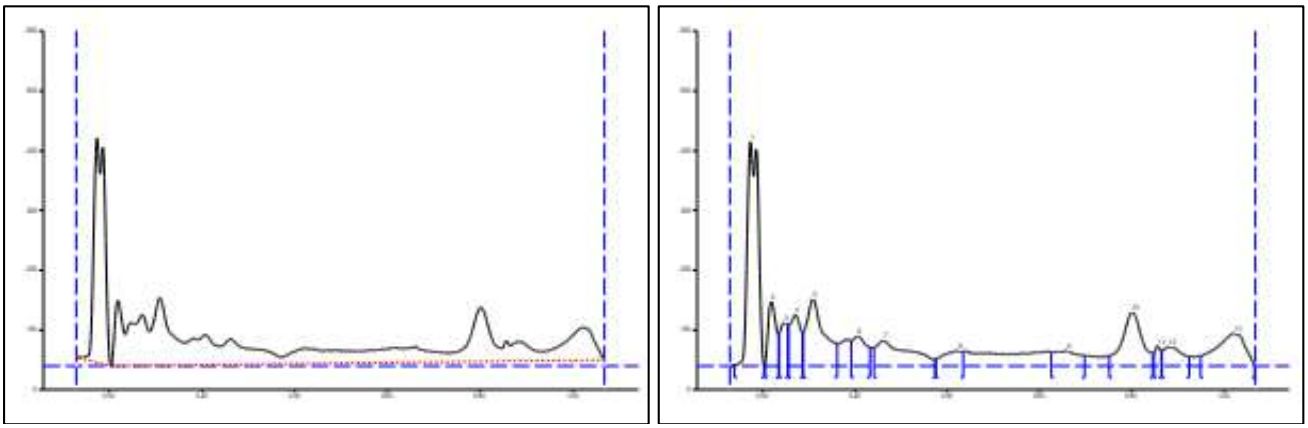

Fig 9: Wave length of compounds of Sample-2 at 366nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.06	2.0	-0.03	375.9	33.67	0.00	0.9	7043.2	25.23
2	0.01	4.3	0.02	109.5	9.81	0.03	53.2	1495.8	5.36
3	0.04	54.6	0.05	72.5	6.49	0.06	69.7	1016.3	3.64
4	0.06	69.8	0.07	86.0	7.70	0.09	56.9	1624.7	5.82
5	0.09	57.1	0.11	113.6	10.17	0.16	37.8	3529.7	12.64
6	0.19	43.1	0.21	50.3	4.51	0.23	31.8	1234.9	4.42
7	0.24	31.4	0.26	43.3	3.88	0.38	11.6	2558.5	9.16
8	0.38	12.1	0.43	25.9	2.32	0.44	24.8	886.7	3.18
9	0.63	23.4	0.66	26.8	2.40	0.70	17.9	1171.0	4.19
10	0.75	17.3	0.80	90.7	8.12	0.85	22.0	3100.2	11.10
11	0.85	22.0	0.86	34.3	3.07	0.87	25.8	345.7	1.24
12	0.87	26.4	0.88	32.5	2.91	0.93	16.1	1086.9	3.89
13	0.95	17.3	1.03	55.1	4.94	1.07	1.8	2825.5	10.12

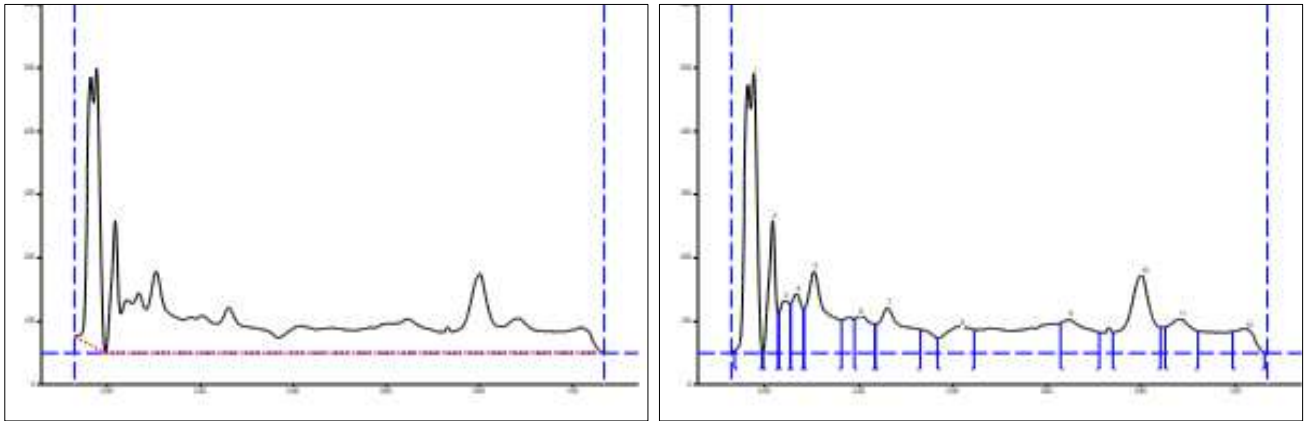

Fig 10: Wave length of compounds of Sample-3 at 366nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.06	4.0	-0.02	441.9	31.47	-0.01	3.9	8896.2	24.17
2	-0.00	0.9	0.02	209.6	14.93	0.03	62.4	2396.9	6.51
3	0.03	62.9	0.04	84.1	5.99	0.05	78.3	1407.4	3.82
4	0.06	78.5	0.07	94.7	6.74	0.08	69.5	1675.9	4.55
5	0.08	69.6	0.11	129.5	9.23	0.16	52.5	4472.6	12.15
6	0.19	54.5	0.20	59.1	4.21	0.23	46.2	1654.7	4.50
7	0.24	46.1	0.26	71.7	5.11	0.33	37.3	3346.5	9.09
8	0.37	23.6	0.42	42.6	3.04	0.45	36.8	1997.2	5.43
9	0.63	47.4	0.65	53.4	3.80	0.71	32.7	2572.1	6.99
10	0.74	32.5	0.80	124.4	8.86	0.84	41.4	4830.3	13.12
11	0.85	40.9	0.88	53.7	3.82	0.92	34.3	2238.2	6.08
12	1.00	34.8	1.02	39.2	2.79	1.07	0.1	1322.0	3.59

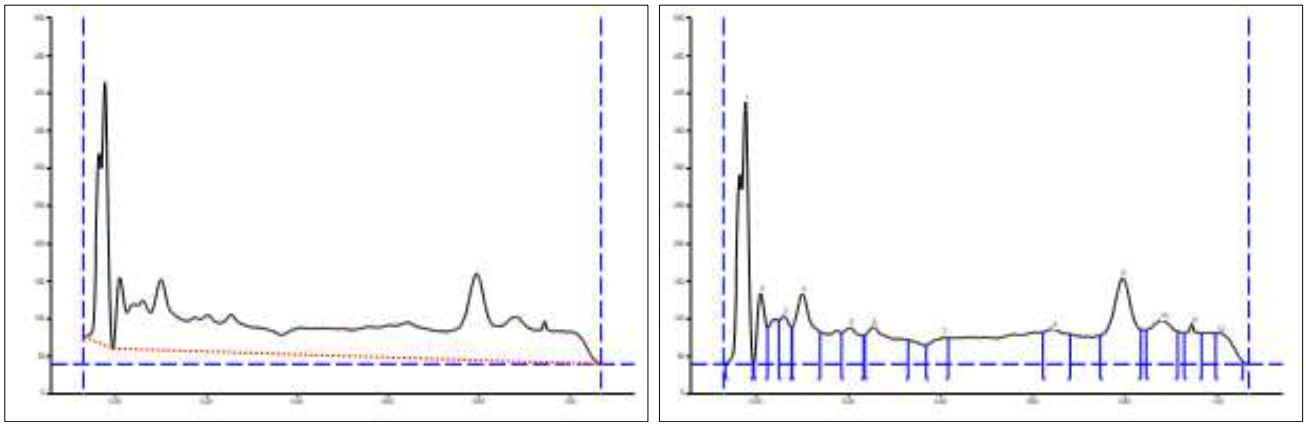

Fig 11: Wave length of compounds of Sample-4 at 366nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.07	0.8	-0.02	349.3	33.13	-0.01	2.3	5594.3	20.47
2	-0.00	2.1	0.01	94.3	8.95	0.02	48.5	1281.5	4.69
3	0.05	58.4	0.06	65.0	6.16	0.08	49.5	1233.8	4.51
4	0.08	49.7	0.10	93.6	8.88	0.14	43.0	2775.1	10.15
5	0.19	42.1	0.20	48.5	4.60	0.23	38.2	1470.0	5.38
6	0.24	39.2	0.26	50.5	4.79	0.33	32.3	2605.9	9.53
7	0.37	25.2	0.41	35.8	3.40	0.42	35.0	1087.7	3.98
8	0.62	42.9	0.65	47.4	4.50	0.68	39.9	1880.0	6.88
9	0.75	38.4	0.80	114.7	10.88	0.84	46.2	4459.4	16.32
10	0.85	45.2	0.88	58.9	5.59	0.92	44.2	2485.3	9.09
11	0.93	41.9	0.95	54.4	5.17	0.97	41.6	1254.6	4.59
12	1.00	40.9	1.00	41.7	3.95	1.06	4.5	1203.1	4.40

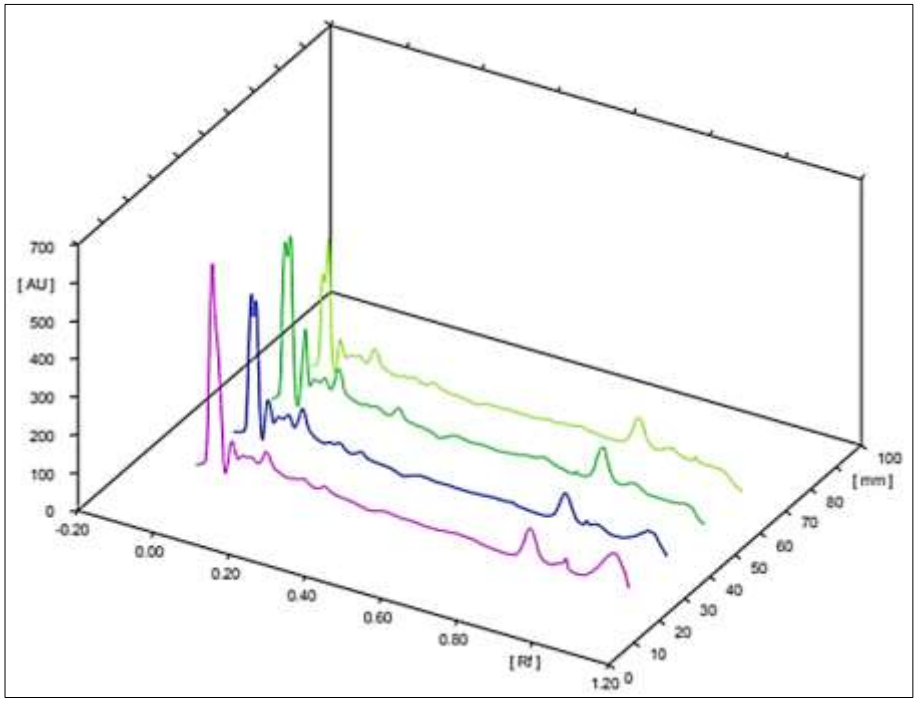

Fig 12: All tracks at Wavelength 366nm

Table 3: Rf value of 4 samples of Shatapushpa at different peaks in HPTLC

Sl no	Peak	Sample 1	Sample 2	Sample 3	Sample 4
1.	1	0. 02	0. 02	0. 02	0. 01
2.	2	0. 05	0. 05	0. 04	0. 06
3.	3	0. 08	0. 07	0. 07	0. 10
4.	4	0. 11	0. 11	0. 11	0. 20
5.	5	0. 21	0. 21	0. 20	0. 26
6.	6	0. 27	0. 26	0. 26	0. 41
7.	7	0. 43	0. 43	0. 42	0. 65
8.	8	0. 65	0. 66	0. 65	0. 80
9.	9	0. 81	0. 80	0. 80	0. 88
10.	10	0. 90	0. 86	0. 88	0. 95
11.	11	-	0. 88	-	-

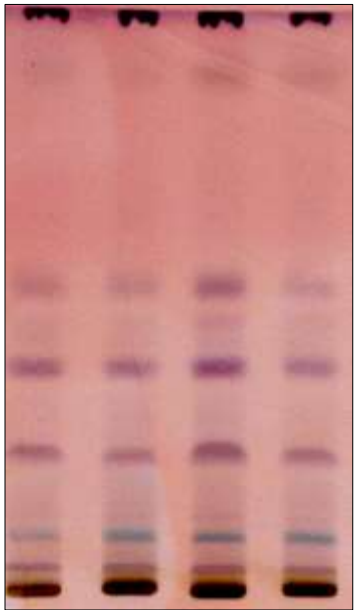

Fig 13: Under visible light

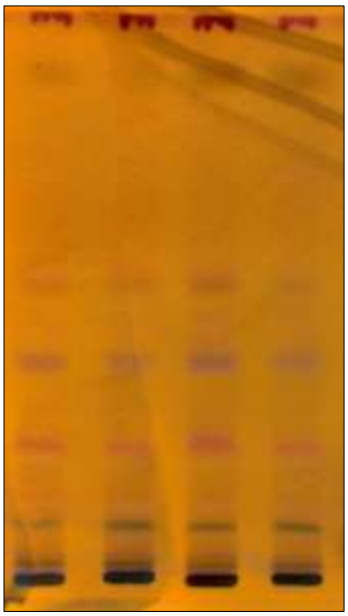

Fig 14: Under 366nm

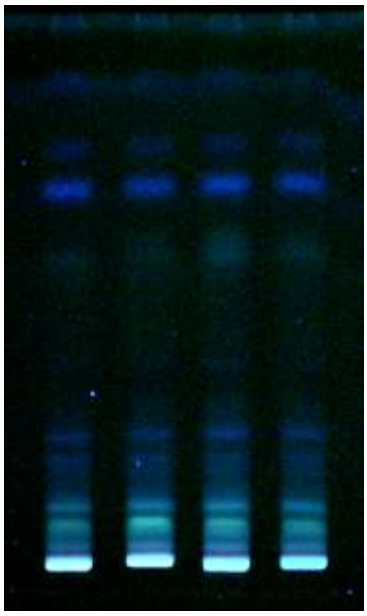

Fig 15: After derivatized

Discussion

Discussion about selection of drug

In classical literature certain rules has been emphasized for the collection of raw drugs. Usually the drugs collected were used in dry form as a part of preservation. But in Sarangadhara Samhita and Bhavaprakasha Samhita certain drugs are told to be used in fresh form only.

Now a days due to lac of knowledge these rules were violated. Shatapushpa is one among the drug which has volatile principles & is an ingredient of many classical preparations. So Shatapushpa was selected for the study that whether drying has any influence on its constituents.

Discussion about selection of 3 different drying methods

By doing literature search it was found that different drying methods influence the quality of drugs having volatile principles. As volatile constituents are thermoliable shade drying was selected as one of the drying method. In classical literature sunlight drying is advised so it also be selected. Hot air oven drying method is a standard drying process under controlled time and temperature. so this method was selected as third method of drying.

Discussion about pharmacognostical analysis

To establish the quality and reliability of the drug, various Pharmacognostic screening measures were adopted. The macroscopic and microscopic features of the collected samples were compared with that of the *description* of Shatapushpa(*Anethum sowa* KURZ) in Ayurvedic Pharmacopoeia of India (API) and Quality standards of medicinal plants for its authentication.

While considering the macroscopical features of the drug, it was found that the fruit of *Anethum sowa* KURZ possess two mericarp with 5 lighter coloured primary ridges of which 3 dorsal, slightly raised, brown, filiform and inconspicuous, 2 lateral prolonged into thin, yellowish membranous wings. Two mericap attached together by a carpophore stalk.

In microscopical features, presence of pericarp, mesocarp, endocarp, 4 vittae, carpophore, aleurone grains in endosperm and vascular strands were helped in the confirmation of the drug identity. These results obtained from pharmacognostical analysis of *Anethum sowa* KURZ assure the genuinity of the drug.

Discussion on Physicochemical analysis

To establish the quality and purity of the drug, physico chemical parameters of four samples of Shatapushpa were analysed and compared with standards mentioned in Ayurvedic Pharmacopoeia of India. All the physico chemical parameters like foreign matter, total ash, acid insoluble ash, water extractives and alcohol extractives comply with API standards. The 4 samples doesn't possess any foreign matters. The % of LOD was more in sample 2(Shade dried fruits of Shatapushpa) . It suggest that the thermoliable compounds were more present in Sample 2(Shade dried fruits of Shatapushpa). The pH of all the samples were almost around 7 indicate that fruits of Shatapushpa is neither acidic nor alkaline in nature. The percentage of total ash & acid insoluble ash were satisfactory with API standards. The water soluble constituents were more in sample 4(Hot air oven dried fruits of Shatapushpa) as it possess more % of water soluble extractive value compared to other samples & alcohol soluble constituents were more in sample 3(Sunlight dried fruits of Shatapushpa) as they possess more % of exrtracive values than other samples.

Discussion on Phytochemical analysis

When the qualitative assessment of 4 samples of Shatapushpa were done, it was found that Dragendorff's test, ferric chloride test, and picric acid test were postitive in all the 4 samples, indicate the presence of alkaloids, phenols and glycosides respectively. At the same time Shinoda's test, Benedicts test & foam test were negative in 4 samples suggest the absence of flavonoids, saponins & carbohydrate accordingly. But tannin test is positive in sample 2 & 4(shade dried and oven dried fruits of Shatapushpa) and negative in sample 1&3. (fresh & sunlight fruits of Shatpushpa) In sample 2& 4 presence of psudotannins may confirm the test as positive. This may be due to the influence of different drying processes.

Discussion on HPTLC

Main constituents of Shatapushpa are di &tri terpenes. so here we selected toluene:chloroform: methanol as mobile phase for HPTLC which fascilitate easy separation of terpenoids. The methanolic extract of fruits of Shatapushpa, sample 1, 2, 3 & 4 evidenced 10, 11, 10 &10 spots respectively. The Rf value corresponding to each spot is given in Table No. 3. The Rf value of all the 4 samples does not show significant differences. The size and number of bands obtained in HPTLC plate was also similar. under visible light, UV 366nm and after dervatization. It suggest that drying process doesn't influence the constituents which was separated through HPTLC method.

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