PHARMACOGNOSTICAL AND PHYTO-CHEMICAL STANDARDIZATION OF VARUNADI CHOORNA - A POLYHERBAL FORMULATION

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ABSTRACT

Introduction: Uterine fibroids do not have definite medical treatment in the modern gynaecological practices other than surgery. When we consider about the present scenario of this disease which is needed to find out appropriate and effective solution of this problem. So many formulations are advised in Ayurveda classics under the Granthi Arbuda and Apachi Chikitsa along with some specific lifestyle restrictions. Ayurveda texts have described Mamsaja granthi which perfectly correlates with benign neoplasm on modern lines. Granthi when present in yoni (female reproductive system) Garbhashaya (uterus) will lead to disturbed menstrual cycle-menorrhagia, metrorrhagia, dysmenorrhea, etc., along with infertility. Most of the Ayurvedic drugs are very effective but lack standardisation. Varunadi decoction is mentioned in Sri Lankan Ayurveda Pharmacopeia which is commonly used by Sri Lankan Traditional Medical Practitioners for Garghasayagata Granthi/Arbuda. Varunadi choorna was selected as the trial drug for uterine fibroid. Aim: To develop the pharmacognostical and phytochemical profile of Varunadi choorna. Material and Methods: Varunadi choorna was prepared as per classical methods and analytical findings were systematically recorded. The samples were subjected to organoleptic analysis, physicochemical analysis and HPTLC examination by optimizing the solvent systems. Results and Conclusions: Pharmacognostical profile of Varunadi choorna was established. The presence of Stone cells, cork cell in surface view, cluster crystal,
lignified stone cells. Pitted stone cell with greenish content, Rhomboidal crystal were the characteristic features observed in the microscopy of the finished product. Physico-chemical analysis showed Loss on drying 0.02% w/w, ash value 0.91% w/w, and pH- 6.5. Water soluble extract 12.50 % (w/w), HPTLC fingerprinting profile of Varunadi choorna revealed 10 spots at 254 nm and 5 spots at 366 nm.

**KEYWORDS:** Organoleptic, Pharmacognosy, HPTLC, Uterine fibroid, Varunadi choorna, Physio-chemical.

**INTRODUCTION**

Uterine fibroid, a noncancerous growth of the uterus also known as fibromyomas, leiomyomas or myomas,[1,2] that often appear during childbearing age (between 35-45 years) in nulliparous or in those having one child infertility of female. Approximately 1.6 million women are newly diagnosed with uterine fibroid per year only in U.S. As a consequence of these local pressure effects and bleeding, uterine fibroids rank as a major reason for hysterectomy accounting for approximately one-third of all hysterectomies or about 2,00,000 hysterectomies per-year.[3,4] The prevalence of uterine fibroids among women between 30-50% in India.[5,6] Uterine fibroids do not have definite medical treatment in the modern gynaecological practices other than surgery, available treatment protocol in modern are hormonal therapy, hysterectomy, myomectomy, myolysis, endometrial ablation and uterine artery embolization having so many complications and expensive also. Ayurveda texts have described Mamsaja granthi[7] which perfectly correlates with benign neoplasm on modern lines. Granthi when present in yoni (female reproductive system) Garbhashaya (uterus) will lead to disturbed menstrual cycle-menorrhagia, metrorrhagia, dysmenorrhea, etc., along with infertility. The aetiology, classification, pathogenesis, and management of Garanthi/Arbuda are discussed at length and in detail in the Ayurveda texts. Mamsa Granthi/Arbuda uterine fibroid is a Bahu Dosh janya disease which involve the Astama Ashaya (Grabhashya) and deep Dhatu in the body. Varunadi decoction is mentioned in Sri Lankan Ayurveda Pharmacopeia which is commonly used by Sri Lankan Traditional Medical Practitioners for Garbhhasayagata Granthi/Arbuda. Standardization is needed to establish quality control parameters for each traditional drug before it is released for use without the fear of toxicity and contamination.[8] So the present work comprises of Pharmacognostical, physiochemical and HPTLC fingerprinting profile for Varunadi choorna.
MATERIALS AND METHODS

Collection, identification and authentication of raw drugs

The raw materials were procured from the pharmacy of Gujarat Ayurved University, Jamnagar. The raw drugs were identified and authenticated for quality and purity in the Pharmacognosy laboratory, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar.

Ingredients of Varunadi choorna

<table>
<thead>
<tr>
<th>Drug</th>
<th>Botanical name</th>
<th>Parted used</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varuna</td>
<td><em>Crataeva nurvala</em> Buch. -Ham</td>
<td>Twak</td>
<td>16g</td>
</tr>
<tr>
<td>Gokshura</td>
<td><em>Tribulus terrestris</em> Linn</td>
<td>Whole plant</td>
<td>16g</td>
</tr>
<tr>
<td>Shunti</td>
<td><em>Zingiber officinale</em> Rosc</td>
<td>Rhizome</td>
<td>16g</td>
</tr>
<tr>
<td>Yavakshara</td>
<td><em>Hordeum vulgare</em> Linn</td>
<td>Whole plant</td>
<td>2g</td>
</tr>
</tbody>
</table>

Preparation of Drug

All 3 ingredients (part of use) will be cleaned and then dried. They will be taken in equal proportion, i.e 48gm will be prepared coarse powder and store in dry container. Yavakshara 2g as a Prakshepa. Yavakshara will be prepared by according to the Kshara preparation method and store as a powder in dry container.

Pharmacognostical Study of Varunadi choorna

Pharmacognostical analysis of the Varunadi choorna was carried out in the Pharmacognosy laboratory of I.P.G.T. & R.A., G.A.U., Jamnagar. It was carried out in two steps.

Organoleptic Study

The organoleptic characters of Varunadi choorna i.e. Color, Touch, Odor and Taste were analyzed with the help of sense organs[9] (Darshana, Sparshana, Aaghrana and Rasana Pareeksha mentioned in Ayurveda).

Microscopic Study

Powder Microscopy Small quantity from the Varunadi choorna was dissolved in distilled water. Few drops of this is spread on a glass slide and covered with a cover slip and excessive water was removed with filter paper. Microscopic examination was done with the prepared slide first without staining and then stained with Phloroglucinol and concentrated HCl under Carl Zeiss Trinocular microscope. Photomicrographs were taken by using Carl Zeiss Trinocular research microscope attached with camera.[10]
Pharmaceutical Study of Varunadi choorna

Physico-chemical Analysis Varunadi choorna was analyzed using various standard physico-chemical parameters such as Loss on drying, Ash value, Water soluble extract, Alcohol soluble extract and pH.\textsuperscript{[11]}

High Performance Thin Layer Chromatography (HPTLC)

HPTLC was performed as per the guideline provided by API. Methanolic extract of drug sample was used for the spotting. HPTLC was performed using Toluene+ Ethyl acetate+ Diethyl amine (7:2:1) solvent system and observed under visible light. The colour and Rf values of resolved spots were noted.\textsuperscript{[12]}

RESULTS AND DISCUSSION

Organoleptic characteristics of Varunadi choorna

Organoleptic characteristics of Varunadi choorna like Colour, Touch, Odour and Taste were recorded and shown in Table No.2.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Characters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Colour</td>
<td>Light brownish</td>
</tr>
<tr>
<td>2</td>
<td>Odour</td>
<td>Slightly aromatic</td>
</tr>
<tr>
<td>3</td>
<td>Taste</td>
<td>Astringent</td>
</tr>
<tr>
<td>4</td>
<td>Touch</td>
<td>Coarse powder</td>
</tr>
</tbody>
</table>

Microscopic Characteristics of Varunadi choorna

Diagnostic characters of Varunadi choorna were observed under the microscope and presence of all ingredients showed their different characters such as Rhomboidal crystal, cluster crystal, lignified stone cells of Varuna, Starch grain, fragment of angular end pitted vessel of Shunti, Stone cells, cork cell in surface view of Varuna, Stone cells, epicarp cells, group of lignified stone cells of Gokshura, Epidermal cells, fibers of Yava Pitted stone cell with greenish content of Varuna, Oleo resin, group of fiber, lignified pitted vessels, lignified simple of Shunti Starch grains & aleurone grains, epidermal parenchyma cells of Yavakshara.

Physico-chemical analysis

Physico-chemical analysis of Varunadi choorna revealed the values such as Loss on drying 0.02% w/w, ash value 0.91% w/w, and pH- 6.5, Water soluble extract 12.50% (w/w), Alcohol soluble extract 9.50% (w/w), and shown in Table No.3.
Table 3: Physico-chemical analysis: Varunadi choorna.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Test</th>
<th>Varunadi choorna</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Loss on drying</td>
<td>0.020 % (w/w)</td>
</tr>
<tr>
<td>2</td>
<td>Water soluble extract</td>
<td>12.50 % (w/w)</td>
</tr>
<tr>
<td>3</td>
<td>Alcohol soluble extract</td>
<td>9.50% (w/w)</td>
</tr>
<tr>
<td>4</td>
<td>pH</td>
<td>6.5</td>
</tr>
</tbody>
</table>

High performance thin layer chromatography of Varunadi choorna

HPTLC Study The chromatographic study (HPTLC) was carried out under densitometer at 254 nm and 366 nm UV to establish fingerprinting profile. Chromatogram shows 10 prominent spots at 254 nm with maximum Rf value 0.03, 0.17, 0.35, 0.39, 0.48, 0.66, 0.73, 0.75, 0.93, 0.98 and 5 spots at 366 nm with maximum Rf value 0.03, 0.17, 0.37, 0.53, 0.98.

Table 4: High performance thin layer chromatography of Varunadi choorna.

<table>
<thead>
<tr>
<th>Wave length /UV</th>
<th>Number of Spots</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 254 nm</td>
<td>10</td>
<td>0.03, 0.17, 0.35, 0.39, 0.48, 0.66, 0.73, 0.75, 0.93, 0.98</td>
</tr>
<tr>
<td>At 366 nm</td>
<td>05</td>
<td>0.03, 0.17, 0.37, 0.53, 0.98.</td>
</tr>
</tbody>
</table>

Plate 1: Microphotographs of Varunadi choorna.

Fig 1: Epicarp cells of Gokshura. Fig 2: Stone cells of Varuna.

Fig 3: Stone cells of Gokshura. Fig 4: Starch grains of Shunti.
Fig 5: Scleroid of Varuna
Fig 6: Scalariform vessels of Shunti

Fig 7: Rosette crystal of Varuna
Fig 8: Prismatic crystal of Varuna

Fig 9: Oloresine contant of Shunti
Fig 10: Group of stone cells of Varuna

Fig 11: Group of Stone cells of Gokshura
Fig 12: Fibres of Yavakshara
**Fig 13:** Fibres of Varuna

**Fig 14:** Epidermal cells of Yavakshara

**Plate 2:** Densitogram of *Varunadi choorna* at 254 nm and 366 nm.

**Plate 2:** Three Dimensional HPTLC (3D) Densitogram.

**Fig 1:** 254nm Peak display.

**Fig 2:** 366nm Peak display.
Varunadi choorna is a formulated combination, consist of four herbal ingredients which were proved to be genuine by assessing the pharmacognostical parameters. The therapeutic effect depends on the quality of the drug administered. All the physico-chemical parameters analyzed were found to be within the normal reference range. Evaluation of physico-chemical parameters and qualitative analysis helped to identify the presence of specific ingredients in a formulation and application of chromatographic techniques aid in recognition of number of ingredients and also to assess the purity by comparing with the standard ones.

**CONCLUSION**

As Varunadi choorna is a formulated combination and intended to given for patients who have uterine fibroids, it is necessary for the authentication of ingredients and standardization to ensure the quality control. Pharmacognostical characteristic of Varunadi choorna under the microscope showed characters of all the ingredients of finished product and there is no major change in the microscopic structure of the raw drugs during the pharmaceutical processes of preparation of the drug. The physicochemical analysis is inferred that the formulation meets maximum qualitative standards and all the parameters discussed here may be used as identifying tools for the quality assessment of this formulated yoga, enabling the reproducibility of the formulation.

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