

PHARMACOGNOSTICAL AND PHARMACEUTICAL EVALUATION OF *SAPTAVIMSHATI GUGGULU* IN THE MANAGEMENT OF ADENOMYOSIS

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ABSTRACT

Background: *Saptavimshati Guggulu* is indicated in cough, asthma, inflammation, hemorrhoids, fistula, angina, flank pain, abdominal colic, proctalgia, nephrolithiasis, dysuria, infection, chronic fever, emaciation, abdominal distention, mental disturbance, skin diseases, all types of sinus wound, *Dushta vrana*, diabetes and filariasis. Considering adenomyosis as an inflammatory state as there is myohyperplasia surrounding the ectopic endometrial glands and stroma in myometrium, leading to bulky uterus, *Saptavimshati Guggulu* along with other therapy has been used to cure this disease. It can also be related to *Dushta vrana* as there is bleeding from the

endometrial cells in the ectopic sites and adenomyosis is related to tissue injury and healing. *Saptavimshati Guggulu* has both anti-inflammatory and wound healing properties as it is indicated in *Sotha* (inflammation) and *Dusta vrana*, it has been used to treat adenomyosis. So for assurance of quality of herbal compounds, pharmacognostical and pharmaceutical analysis of the drug should be done. **Methods:** *Saptavimshati Guggulu* was subjected to microscopic evaluation for pharmacognostical, physiochemical analysis like weight variation, loss on drying, ash value, pH value, water soluble extract, alcohol soluble extract and high-performance thin layer chromatography (HPTLC). **Results:** Pharmacognostical study showed the presence of certain identifying characters of the ingredients of *Saptavimshati Guggulu* i.e., *Triphala*, *Trikatu*, *Giloya*, *Musta*, *Chitraka*, *Kachur*, *Ela*, *Vidanga*, etc. In pharmaceutical study, preliminary physiochemical analysis showed mean weight .469gm, pH value 5 (by pH meter at room temperature), ash value 9.5% w/w, loss on drying 12.3% w/w, water soluble extract 59.3% w/w, alcohol soluble extract 25.4% w/w and HPTLC showed 6 spots in 254 nm

and 4 spots in 366 nm. **Conclusions:** Pharmacognostical and physico-chemical observations revealed the specific characters of all active constituents of *Saptavimshati Guggulu* and confirmed the purity and genuinity of the drug.

KEYWORDS: Adenomyosis, Pharmaceutical analysis, Pharmacognosy, *Saptavimshati Guggulu*.

INTRODUCTION

Saptavimshati Guggulu is given in *Bhagandara roga Chikitsa* by *Chakradutta*.^[1] It is indicated in cough, asthma, inflammation, hemorrhoids, fistula, angina, flank pain, abdominal colic, proctalgia, nephrolithiasis, dysuria, infection, chronic fever, emaciation, abdominal distention, mental disturbance, skin diseases, all types of *Naadi vrana* (sinus wound), *Dushta vrana*, diabetes and filariasis. It is also mentioned in *Yogaratanakara Sadhyovrana Chikitsa*.^[2] Adenomyosis is a condition where there is ingrowth of the endometrium, both the glandular and stromal components, directly into the myometrium.^[3] Women affected by adenomyosis may present with abnormal uterine bleeding (AUB), dysmenorrhea, dyspareunia, or infertility but one third of them are asymptomatic.^[4] Although the pathogenesis is still unclear, uterus basement membrane invagination caused by endometrial tissue damage and repair, and embryonic stem cell residue and differentiation, are the two existing theories of adenomyosis^[5] at present. The former considered that adenomyosis is mainly associated with high hormone levels,^[6] tissue damage, and repair,^[7] the latter believed that adenomyosis is caused by an embryonic remnant or adult stem cell differentiation.^[8] Chronic peristaltic myometrial contractions may induce continuous microtrauma to the JZ, causing inflammation which in turn promotes local increased estrogen production, inducing a vicious cycle. A positive feedback mechanism is generated, and chronic hyperperistalsis in the JZ promotes repeated cycles of autotraumatization.^[9] Considering adenomyosis as an inflammatory state as there is myohyperplasia surrounding the ectopic endometrial glands and stroma in myometrium, leading to bulky uterus.^[10], *Saptavimshati Guggulu* along with other therapy has been used to cure this disease. It can also be related to *Dushta vrana* as it is associated with tissue damage and repair. Also, there is bleeding from the endometrial cells in the ectopic sites during menses. *Saptavimshati Guggulu* has both anti-inflammatory and wound healing properties as it is indicated in *Sotha* (inflammation) and *Dusta vrana*. It is also mentioned in *Kukshi Ruja & Basti Ruja* which helps in relieving dysmenorrhea which is a major symptom of adenomyosis.

MATERIALS AND METHODS

Collection, identification and authentication of raw drugs.

The raw materials were procured from the pharmacy of ITRA, Jamnagar and some none available drugs were collected from the local market of Jamnagar. They all were identified and authenticated in the pharmacognosy laboratory of ITRA, Ministry of Ayush, Govt. of India, Jamnagar before manufacture of medicine. The ingredients and part used of the *Saptavimsati Guggul* are given in Table 1.

The final product i.e., *Saptavimsati Guggul* was prepared at Dept of *Rasa Shastra & Bhaishajya Kalpana* Laboratory of I.T.R.A, Jamnagar. Once the formulation was ready, it was subjected to pharmaceutical evaluation and HPTLC at pharmaceutical laboratory, I.T.R.A, Jamnagar.

Method of preparation

First of all, *Guggul* was broken into small pieces, water was added in *Guggul* to washout foreign matters manually and filtered. *Triphala yavakuta* (*Amalaki*, *Bibhitaki* & *Haritaki* = 1:1:1) 1 part was treated with 16 parts water and a decoction was made by boiling the mixture until its volume remains one eighth. It was filtered through a muslin cloth. Then it was kept in a big vessel by adding *Triphala Kwath* (*Guggul*: *Triphala Kwath*=1:8) and filtered again. It was kept as it is for 24 hours then boiled and let all water to evaporate in mild heat.^[11] After *Sodhana* of *Guggul* was finished, fine powder (passed through sieve no 44) of all the drugs were added in it as per mentioned in table no. 1. A dough suitable for *Vati* preparation was made by mixing them well & kneaded. Small tablets of approx. 500mg each were prepared with the dough rolling manually by applying ghee and left for drying. After that, prepared *Guggulu* was stored in air tight container under hygienic condition.

Table 1: Ingredients of *Saptavimsati Guggul* (*Chakradatta- Bhagandar Chikitsa* 46/13-18).

| CONTENTS | LATIN NAME/ ENGLISH NAME | PART USED | FORM | RATIO |
|---------------|--|-------------------|--------|-------|
| 1.Haritaki | <i>Terminalia chebula</i> Retz. | Dried Fruit | Powder | 1part |
| 2.Bibhitaki | <i>Terminalia belerica</i> (Gaertn.) Roxb. | Dried Fruit | Powder | 1part |
| 3.Amalaki | <i>Emblca officinalis</i> Gaertn | Dried Fruit | Powder | 1part |
| 4.Musta | <i>Cyprus rotundus</i> L. | Dried Rhizome | Powder | 1part |
| 5.Vayuvidanga | <i>Emblca ribes</i> Burm.f. | Dried Fruit | Powder | 1part |
| 6.Giloya | <i>Tinospora cordifolia</i> Willd. | Dried Stem | Powder | 1part |
| 7.Chitrakmool | <i>Plumbago zeylanica</i> L. | Dried Root Bark | Powder | 1part |
| 8.Kachur | <i>Curcuma zedoaria</i> (Christm.) Roscoe | Dried Rhizome and | Powder | 1part |

| | | | | |
|--------------------------------|--|--------------------|--------|---------|
| | | Leaves | | |
| 9.Elaichi | <i>Elettaria cardamomum</i> (L.) Maton | Dried Fruit | Powder | 1part |
| 10.Piplimool | <i>Piper longum</i> Linn. | Dried Root | Powder | 1part |
| 11.Haauber/ Hriber | <i>Juniperus communis</i> L. | Dried Seed | Powder | 1part |
| 12.Devadaaru | <i>Cedrus deodara</i> (Roxb.)G.Don | Heart wood | Powder | 1part |
| 13.Tumbaru | <i>Zanthoxylum aromaticum</i> DC. | Dried Fruit | Powder | 1part |
| 14.Pooshkarmool | <i>Inula racemosa</i> Hook.f.J. | Dried Root | Powder | 1part |
| 15.Chabya | <i>Piper chaba</i> Trel. &Yunck | Dried Root | Powder | 1part |
| 16.Vishaalaa (Indravaaruni) | <i>Citrullus colocynthis</i> L. (Schrad) | Dried Root / Fruit | Powder | 1 part |
| 17.Haldi | <i>Curcuma longa</i> L. | Dried Rhizome | Powder | 1 part |
| 18.Daaruhaldi | <i>Berberis aristata</i> DC. | Dried Root | Powder | 1 part |
| 19.Bidalawana | Sedimented salt | - | Powder | 1 part |
| 20.Saubarchal lawan | Black salt | - | Powder | 1 part |
| 21.Yavakshar | <i>Hordeum vulgare</i> L. (Potassium carbonate) | Alkali | Powder | 1 part |
| 22.Sajjikshar | Sodiumb carbonate | Alkali | Powder | 1 part |
| 23.Saindhavlawan | Rock salt | - | Powder | 1 part |
| 24.Gajapippali | <i>Scindapsus officinalis</i> (Roxb.) Schott | Dried Fruit | Powder | 1 part |
| 25.Pippali | <i>Piper longum</i> Linn. | Dried Fruit | Powder | 1 part |
| 26.Shrungver/Shu nithi | <i>Zingiber officinale</i> Roscoe | Dried Rhizome | Powder | 1 part |
| 27.Maricha | <i>Piper nigrum</i> Linn. | Dried Fruit | Powder | 1 part |
| 28.SuddhaGuggul | <i>Commiphora mukul</i> | Resin | - | 54 part |

PHARMACOGNOSTICAL STUDY

The pharmacognostical study was divided in to organoleptic study and microscopic study of the finished product.

➤ Organoleptic Study

The genuinity of the polyherbal formulation can be fined with organoleptic characters of the given sample. Organoleptic parameter comprises taste, color, odor and touch of *Saptavimshati Guggulu* which was scientifically studied as per the standard references.^[12]

➤ Powder microscopy

Powdered drug was studied microscopically and microscopic characters of individual drugs were noted. The powder of the drug was dissolved with water followed by microscopy of the sample without stain and after staining with Phloroglucinol & HCl. Microphotographs of the sample were taken under Carl-Zeiss trinocular microscope.^[13]

PHARMACEUTICAL STUDY

Physico-chemical analysis

With the help of various standard physico-chemical parameters, *Saptavimshati Guggulu*, was analysed. The common parameters mentioned for *Saptavimshati Guggulu*, in Ayurvedic Formulary of India (AFI Part 2, Vol 2 – First Edition)^[14] and CCRAS guidelines^[15] are loss on drying, total ash, water soluble extract, alcohol soluble extract and pH value.

High performance thin layer chromatography

Analysis of pharmaceutical compounds and newer drugs is commonly used in all the stages of drug discovery and development process. These analytical techniques provide more accurate and precise data, not only supporting drug discovery and development but also post market surveillance.^[16] HPTLC is an analytical technique based on TLC, but with enhancements intended to increase the resolution of the compounds to be separated and to allow quantitative analysis of the compounds. Some of the enhancements such as the use of higher quality TLC plates with finer particle sizes in the stationary phase which allow better resolution.^[17]

RESULTS

The initial purpose of the study was to confirm the authenticity the drugs used in preparation of *Saptavimsati Guggul*. For this, all the ingredients were subjected to organoleptic and microscopic evaluations to confirm the genuineness of the raw drugs. Later after the preparation of formulation, pharmacognostical evaluation was carried out. Organoleptic evaluation includes screening of organoleptic characters like colour, odor, taste, etc. of the *Saptavimsati Guggul* (Table no.2).

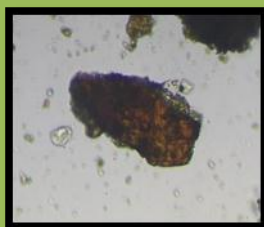
Table 2: Organoleptic characters of *Saptavimsati Guggul*.

| Parameter | Results |
|-------------|---------------------|
| Color | Dark brownish black |
| Odor | Aromatic |
| Taste | Bitter astringent |
| Consistency | Hard solid |

MICROSCOPIC EVALUATION

Microscopic evaluation was conducted by dissolving *Saptavimsati Guggul* in the distilled water and studied under microscope for the presence of characteristics of ingredient drugs. The diagnostic characters are *Pippali*- Bottle neck shaped stone cells, Mesocarp with tannin, Oil content. *Amalaki*-Silica deposition, fibres, scleroids, vessels. *Bibhitaki* - Lignified stone

cells, Rosette crystal, trichome, scleroids, stone cells. *Haritaki*- Scleride of *Haritaki*, Epicarp cells, Mesocarp cells, Starchgrains, Pitted stone cells of *Haritaki*, Tannin of *Haritaki*. *Chavya*- Cork cells, stone cells. *Chitrakmool*- Border pitted vessels of *Chitraka*, lignified border pitted vessels of *Chitraka*, lignified parenchyma cells of *Chitraka*, Tannin content of *Chitraka*. *Daruharidra*- Starchgrains, Scleroids, Prismatic crystal, Pitted veels, Cork cells, Brown content. *Shunti*: olioressin content of *Shunthi*, starch grain of *Shunthi*, *Devdaru*: fibre passing through medillary rays of *Devdaru*, lignified fibre of *Devdaru* with sieve elements, rhomboidal crystals of *Devdaru*, *Ela* - Microcrystals of *Ela*, Striated fibres of *Ela*. *Gajapippali*- Acicular crystals, Pitted vessels. *Guduchi*- Collenchyma cells, Cork cells, Lignified collenchyma cells, Pitted vessels, Starch grains. *Haridra*- Cork in tangential view, Oleoresin content, Parenchyma cells, Scalariform vessels. *Kachoor*- Crystals, Fibres, Stone cells. *Marich*- Black debries, Stone cells, Fiber. *Musta*- Cork cells of *Musta*, fibres of *Musta*, Silica depostion of *Musta*. *Pushkara*- Crystals, Pitted vessels, Starch grains. *Tumburu*- Group of fibres, Mesocarp cells, starch grains. *Vidanga*- Scleroids, pool cells, Stone cell, Trichome. *Vishala*- Lignified fibre, Simple trichome, Spiral vessels.



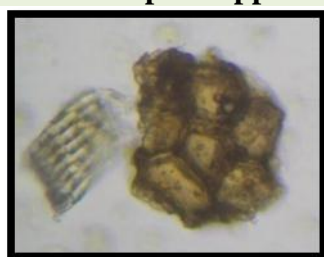
A. Mesocarp of Pippali



B. Scleroid of Amalaki



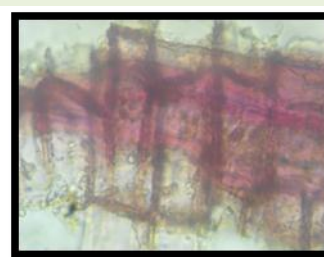
C. Trichome of Bibhitaki



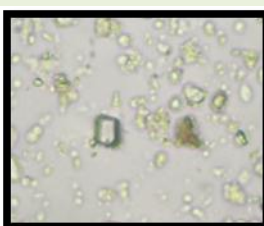
D. Cork cells of Chavya



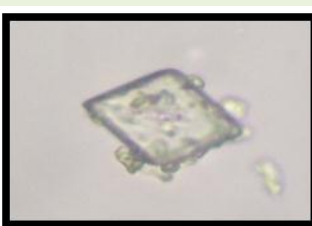
E. Collenchyma of Guduchi



F. Lignified parenchyma cells of Chitraka



G. Rhomboidal crystals of Devdaru



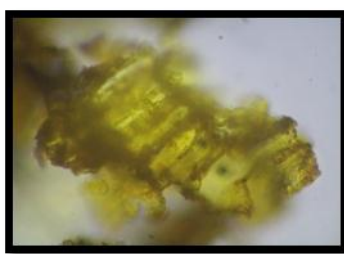
H. Prismatic crystals of Daruharidra



I. Microcrystals of Ela



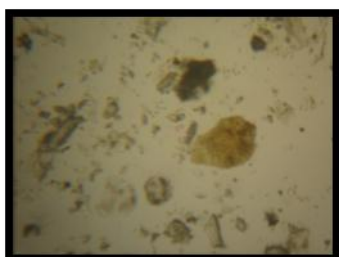
J. Acicular crystals of Gajapippali



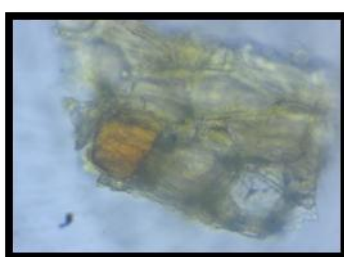
K. Cork in tangential view of Haridra



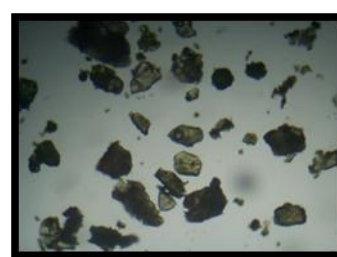
L. Fibers of Kachoor



M. Tannin of Haritaki



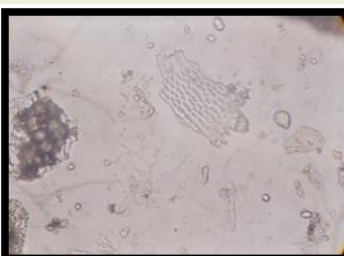
N. Cork cells of Musta



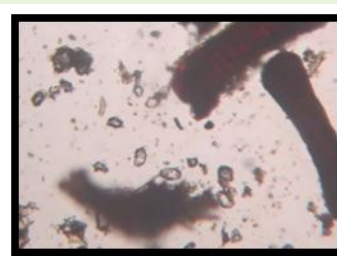
O. Black debris of Maricha



P. Crystals of Pushkara



Q. Fragment of pitted vessel of Shunthi



R. Starch grains of Tumbaru



S. Trichome of Vidanga



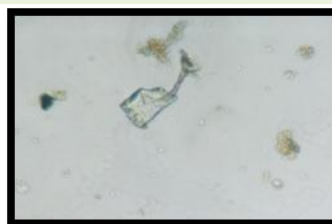
T. Spiral vessels of Vishala



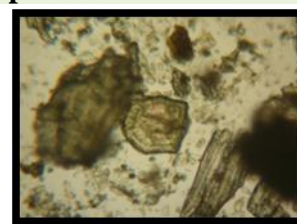
U. Annular vessels of Pippali mool



V. Fiber narrow lumen of Pippali mool



W. Prism cells of Hriber



X. Pitted stone cells of Haritaki

Fig. 1: Microphotographs of *Saptavimsati Guggul*.

Saptavimshati Guggulu was and dissolved with water and microscopy of the sample was done without stain and after staining with phloroglucinol and HCl.

PHYSICO-CHEMICAL PARAMETERS

Physico-chemical parameters like loss on drying, pH values, etc. were found within the normal range. Detail is given in the Table 3.

Table 3: Physico-chemical parameters of *Saptavimshati Guggulu*.

| Parameter | Value |
|-------------------------------|-------|
| Loss on drying (w/w) | 12.3% |
| Total Ash value (w/w) | 9.5% |
| Water soluble extract (w/w) | 59.3% |
| Alcohol soluble extract (w/w) | 25.4% |
| pH (by pH meter) | 5.0 |
| Mean Weight (gm) | .469 |

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

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 (9:1 v/v) as solvent system and observed under visible light. The color and R_f values of resolved spots were noted.^[18]

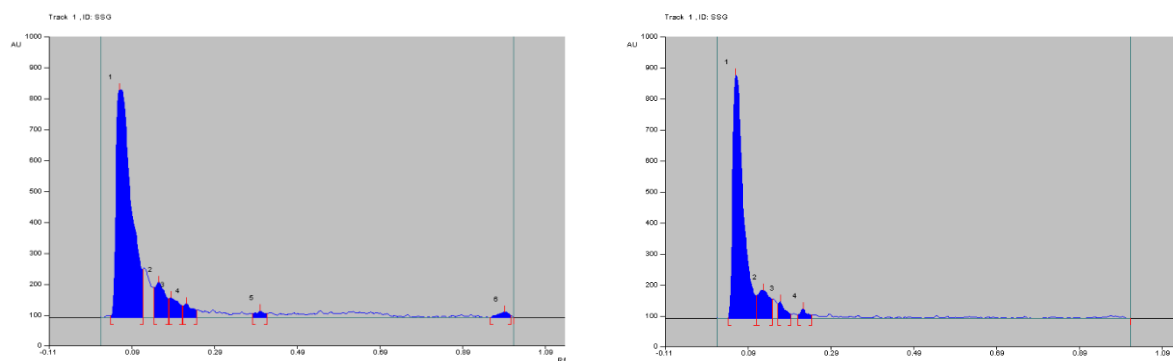
R_f Value (Retention factor value)

The distance of each spot from the point of its application was measured and recorded and the R_f Value was calculated by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.^[19]

HPTLC STUDY

Table No- 4: HPTLC profile/ R_f values of *Saptavimshati Guggulu*.

| Wavelength | No. of Spots | R_f Value |
|-------------------|--------------|------------------------------------|
| Short UV (254 nm) | 6 | 0.06, 0.16, 0.18, 0.22, 0.4, 0.99. |
| Long UV (366nm) | 4 | 0.06, 0.13, 0.17, 0.22 |



Densitometry at 254 nm

Densitometry at 366 nm

Fig. No-3: Densitometry of *Saptavimshati Guggulu*.

DISCUSSION

This confirms the presence of all active ingredients of raw drugs in the final product. All the physico-chemical parameters i.e., Loss on drying, Specific gravity, Acid value, saponification, Iodine Value, Refractive index, pH value etc. were analysed and found to be within the normal reference range. The physicochemical analysis showed pH value 5 (by pH meter at room temperature) which indicates that the drug is slightly acidic, ash value 9.5%w/w, loss on drying 12.3%w/w, water soluble extract 59.3%w/w, alcohol soluble extract 25.4%w/w (water soluble material is more than alcohol soluble material) and HPTLC showed 6 spots in 254 nm and 4 spots in 366 nm.

CONCLUSION

The pharmacognostical and Physico-chemical analysis of *Saptavimshati Guggulu* confirmed the purity and genuinity of the drug. Genuinity of drugs is of utmost importance in present era where there is more adulteration in drug contents. Information acquired from this study may be beneficial for further research work and can be used as a reference standard for quality control researches.

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Conflict of interest: None

Declared Ethical Approval: The study was approved by the Institutional Ethics Committee

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