



**PHARMACOGNOSTICAL AND PHYTO-CHEMICAL
STANDARDIZATION OF KASHMARYADI GHRITA - A HERBAL
FORMULATION FOR RECURRENT ABORTION**

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ABSTRACT

Introduction: In *Ayurveda*, every substance can be used as a drug if used. Though many medicines are mentioned in our ancient Ayurvedic literature, they need to be standardized to ensure their quality, identity, safety and efficacy. Pregnancy is a physiological process of reproduction during which various changes take place in a woman's body. Among various complications seen in pregnancy, abortion (*Garbhasrava/Garbhapata*) is one of the most common problem seen in today's era due to changed lifestyle, diet and stress. *Kashmaryadi Ghrita* is mentioned by *Acharya Charaka* and *Acharya Vagbhata* in

Putraghni and *Raktayoni Yonivyapad* in *Ayurveda* which denote recurrent abortion in modern science. The present work was carried out to standardize the finished product *Kashmaryadi Ghrita* to confirm its identity, quality and purity. **Aim:** To develop the pharmacognostical and phyto-chemical profile of *Kashmaryadi Ghrita*. **Material and Methods:** *Kashmaryadi Ghrita* was prepared as per classical methods and analytical findings were systematically recorded. The samples were subjected to organoleptic analysis, physico-chemical analysis and High performance Thin Layer Chromatography (HPTLC) examination by optimizing the solvent systems. **Results and Conclusions:** Pharmacognostical profile of *Kashmaryadi Ghrita* was established. The presence of prismatic crystals, rhomboidal crystals, brown content, spool cells, epicarp cells, mesocarp cells, simple and compound starch grains,

lignified fibres, lignified stone cells, stone cells, fibres, scleroid, fragment of testa with brown content were the characteristic features observed in the microscopy of the drug combination. Physico-chemical analysis showed refractive index of *Kashmaryadi Ghrita* as 1.4710, specific gravity 0.9096, iodine value 18.086, saponification value 193.308 and acid value 1.5990. HPTLC fingerprinting profile of *Kashmaryadi Ghrita* revealed 7 spots at 254 nm and 1 spot at 366 nm.

KEYWORDS: Organoleptic, Pharmacognosy, Abortion, *Kashmaryadi Ghrita*, Physico-chemical.

INTRODUCTION

In the present scenario, *Ayurveda* has become an important part of our lives as not only the diet and lifestyle mentioned in *Ayurveda* is followed by people but also the various treatment options mentioned in *Ayurveda* including the various single drugs and compound formulations show effective results in various diseases which are untreatable by modern medicine. So in order to meet the ends of demand and supply of *Ayurvedic* formulations, several spurious & substandard drugs are sold in market without proper & abundant cultivation practices.^[1] So to cope up with new challenges to be faced by herbal drug industry & to worldwide acceptance of herbal drugs, standardization of raw drugs, in process drugs & finished products is necessary.^[1] Standardization is a specific property that quantifies the purity and quality of drugs and formulations.

Pregnancy, also known as the gestation, is the time during which one or more offspring develops inside a woman's womb.^[2] The entire period is divided in 3 trimesters which includes 40 weeks. The first trimester i.e. first 12 weeks is the most crucial period as the most common problem seen during this period is abortion which affects the females physically as well as emotionally. Abortion is defined as expulsion or extraction of an embryo or fetus weighing 500 gm or less (before 22 weeks of gestation) when it is not capable of independent survival or before its age of viability.^[3] The term miscarriage is the recommended terminology for spontaneous abortion.^[3] Recurrent abortion is defined as a sequence of 3 or more consecutive spontaneous abortion before 20 weeks.^[4] This distressing problem is affecting approximately 1% of all women of reproductive age.^[4] The risk increases with each successive abortion reaching over 30% after 3 consecutive losses.^[4] The most common causes responsible for spontaneous abortion are faulty development of embryo resulting from chromosomal anomalies(50%), placental abnormalities, endocrinal disturbances(10-15%),

acute infectious diseases(5%), severe trauma and shock. Other causes include anatomical abnormalities (10-15%) like cervical incompetence, congenital malformation of uterus, uterine fibroid, uterine synechiae; immunological factors and use of certain drugs.^[5] In 40-60% cases, cause remain unexplained.^[5]

In *Ayurveda*, the expulsion of fetus upto 4th month of pregnancy is termed as *Garbhasrava* and there after in 5th and 6th month it is termed as *Garbhapata* because by this period the fetal parts have attained some stability as told by *Acharya Sushruta*.^[6] *Charaka* says that abnormalities in the factors responsible for proper growth and development of fetus can cause either intrauterine death of the fetus or its expulsion before viability.^[7] *Charaka* also mentioned *Shukra Dosha*^[8] and *Vata Vikrati*^[9] as responsible factors for *Garbhasrava*. *Bhela* says that all twenty *Yonivyapad* can cause abortion^[10] while *Harita* told vitiated *Doshas* specially *Vata* responsible for expulsion of fetus before 10th month.^[11] In *Bhavaprakasha*, *Garbhanasha* is enumerated as one of the eighty disorders of *Vata*.^[12] Expulsion of fetus from very early or blastocystic stage to second trimester is the main clinical feature of various *Jataharinis* (*Andaghni*, *Durdhara*, *Kalaratri* and *Vasya*).^[13] Similarly, *Yonivyapad* *Putraghni*,^[14,15] *Vamini*,^[16] *Asraja*^[17] denote repeated abortion.

Kashmaryadi Ghrita is the *Ayurvedic* formulation mentioned by *Acharyas* in *Putraghni* and *Raktayoni Yonivyapad*.^[18,19] The present study was done for standardization of *Kashmaryadi Ghrita* through pharmacognostical and pharmaceutical standards.

MATERIALS AND METHODS

Collection of Raw Materials

Raw drugs of *Kashmaryadi Ghrita* were procured from the ground and Pharmacy of Gujarat Ayurved University, Jamnagar. The ingredients were identified and authenticated at Pharmacognosy laboratory of Institute for Postgraduate Teaching and Research in Ayurveda, Gujarat Ayurved University Jamnagar.

Method of preparation of *Kashmaryadi Ghrita*

For the preparation of drug, *Kalka* was taken as one part , ghee was taken 4 times to *Kalka* and *Kwatha* was taken 4 times to ghee.^[20] All the drugs for *Kalka* and *Kwatha* were same and also in equal quantity as mentioned in Table 1. The drug was prepared as per the *Ghrita Paka Vidhi* mentioned in *Sharangdhara Samhita*.

Pharmacognostical Evaluation

Morphological, organoleptic and microscopic evaluation of *Kashmaryadi Ghrita* ingredients were carried out at Pharmacognostical laboratory of institute. Individual powder (fine powder) were dissolved in small quantity of distilled water, was studied under the Carl zeiss trinocular microscope attached with camera, with stain (Phloroglucine and concentrated HCl) and without stain. The microphotographs were also taken under the microscope.^[21,22]

Physico- chemical evaluation

Kashmaryadi Ghrita was analysed by using standard qualitative and quantitative parameters at Pharmaceutical laboratory of institute according to protocol for testing of Ayurveda, Siddha & Unani medicine of *Sneha Kalpana* for Specific gravity, Refractive index, Iodine value, Acid value and Saponification value.^[23]

HPTLC Profile

HPTLC analysis of the formulation was performed by using CAMAG TLC Scanner 3. The stationary phase used was HPTLC plate's Silica gel 60 F254 and mobile phase was Petroleum ether (60-80): Diethyl ether: Acetic acid (9:1:0.1 V/V). The sample was prepared by taking 0.1 ml of sample and diluted it with 1 ml of hexane then used for chromatography. Thereafter, prechromatographic derivatisation was done with alcoholic KOH on plate itself and then heated for 15 min. (alkaline hydrolysis) then the plate was developed. The developed plate was derivatised by 5% sulphuric acid by dipping technique and heated till complete colour development (Ref: Stahl). The plate was visualized under short (254 nm) and long (366 nm) ultraviolet (UV) radiations and density of the separated spots was recorded using scanner III. The R_f values were recorded.^[24]

OBSERVATIONS AND RESULTS

Pharmacognostical Evaluation

Organoleptic Evaluation

Organoleptic parameters like Colour, Taste, Odour and Touch of *Kashmaryadi Ghrita* were scientifically recorded and results are as per mentioned in Table 2.

Microscopic Study

The powder of *Kashmaryadi Ghrita* ingredients when observed under the microscope showed the characters like cork in surface view of *Kutaja*, rhomboidal and prismatic crystals of *Kutaja*, simple and compound starch grains of *Kutaja*, simple, septate and lignified fibres of

Kutaja, lignified stone cells of *Kutaja*, lignified sclroids of *Kutaja*, oil globule of *Kutaja*, tannin content of *Kutaja*, brown content and spool cells of *Gambhari*, epicarp and mesocarp cells of *Gambhari*, stone cells of *Gambhari*, lignified stone cells of *Gambhari*, fibres of *Gambhari*, sclroid of *Gambhari*, fragment of testa with brown content of *Gambhari* as depicted in plate 1.

Physicochemical analysis

Physico chemical analysis of *Kashmaryadi Ghrita* was carried out which included the parameters like refractive index, specific gravity, iodine value, saponification value and acid value. The results obtained were mentioned in Table 3.

HPTLC

Chromatographic study (HPTLC) was carried out under 254 and 366 nm UV to establish fingerprinting profile. It showed 7 spots at 254 nm and 1 spot at 366 nm. Results of HPTLC are given in Table 4 and densitogram is shown in Plate 2.

Table 1: Ingredients of *Kashmaryadi Ghrita*.

S. No.	Drug	Latin name	Drug proportion	Part used
1.	<i>Kashmari (Gambhari)</i>	<i>Gmelina arborea</i> Roxb.	96 kg	Fruit
2.	<i>Kutaja</i>	<i>Holarrhena antidysenterica</i> (Roxb.ex Flem.)Wall.	96 kg	Stem bark
3.	<i>Go Ghrita</i>	-	45 kg	-

Table 2: Organoleptic characters of *Kashmaryadi Ghrita*.

S. No.	Organoleptic characters	Results
1.	Colour	Greenish yellow
2.	Odour	Characteristic
3.	Touch	Slippery
4.	Taste	Bitter

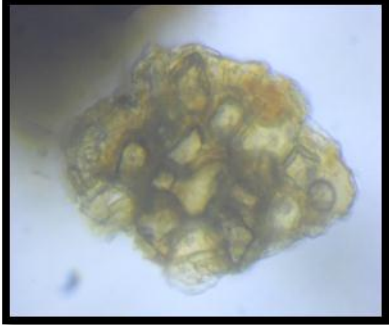
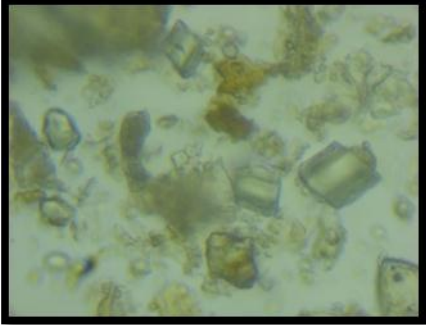
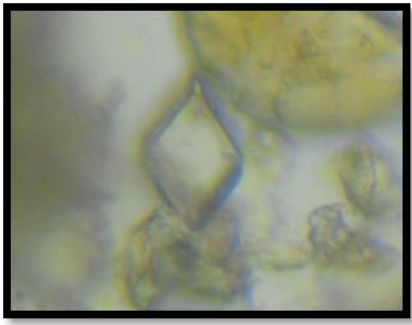

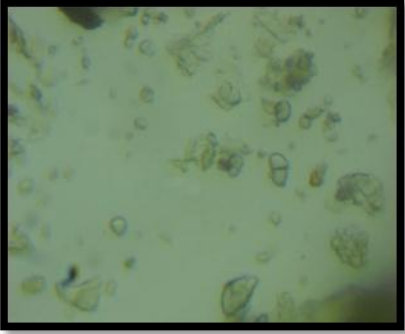




Table 3: Physico- chemical analysis of *Kashmaryadi Ghrita*.

S. No.	Parameters	Results
1.	Specific Gravity (at room temperature)	0.9096
2.	Refractive Index (at room temperature)	1.4710
3.	Acid value (w/w)	1.5990
4.	Iodine value (w/w)	18.086
5.	Saponification value (w/w)	193.308

Table 4: HPTLC Profile of *Kashmaryadi Ghrita*.

Sample	254 nm		366 nm	
	No. of spots	Rf values	No. of spots	Rf values
Kashmaryadi Ghrita	7	0.14,0.21,0.24,0.32, 0.58,0.83,0.97	1	0.01

Plate 1: Microscopic characters of *Kashmaryadi Ghrita*.

<p>Cork in surface view of Kutaja</p> 	<p>Prismatic crystal of Kutaja</p> 	<p>Rhomboidal crystal of Kutaja</p> 
<p>Simple fibre and Prismatic crystal of Kutaja</p> 	<p>Simple & Compound starch grains of Kutaja</p> 	<p>Lignified fibre of Kutaja</p> 
<p>Group of stone cells of Kutaja</p> 	<p>Lignified scleroids of Kutaja</p> 	<p>Lignified stone cell of Kutaja</p> 
<p>Septate fibre of Kutaja</p>	<p>Tannin content of Kutaja</p>	<p>Epicarp cells of Gambhari</p>

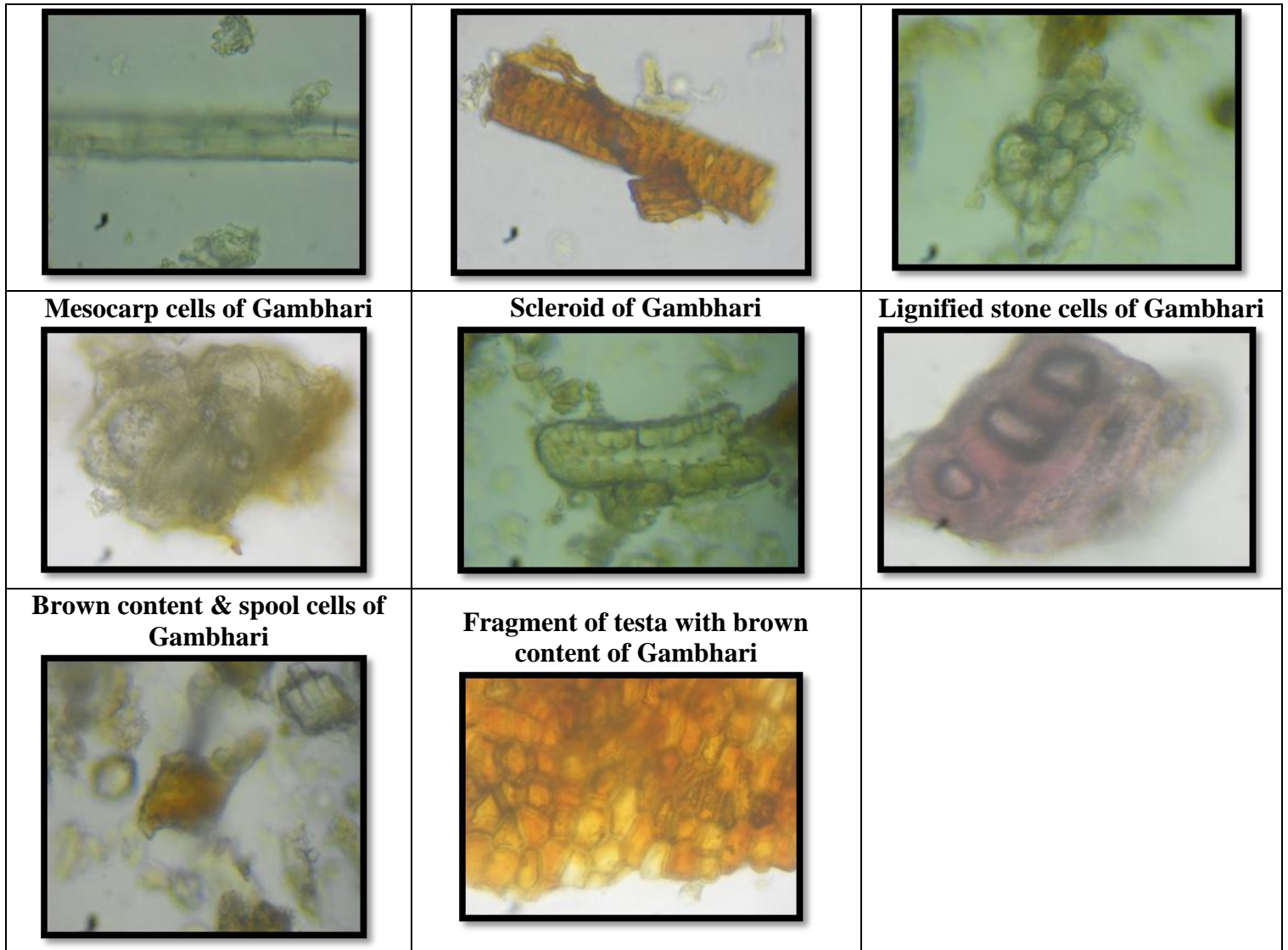
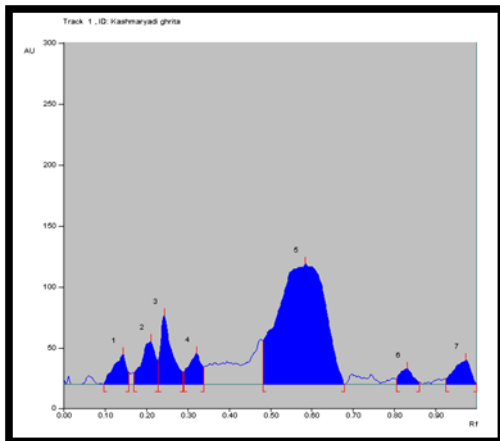
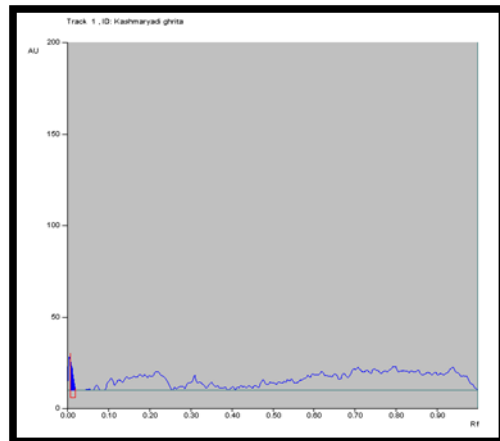


Plate 2: HPTLC Evaluation of *Kashmaryadi Ghrita*



HPTLC at 254 nm



HPTLC at 366 nm

DISCUSSION

Pharmacognostical evaluation showed that *Kashmaryadi Ghrita* contains all the ingredients which were observed in the microscopical characters which proves the purity and quality of the product. Physicochemical analysis showed that material gains no moisture during storage, so quality of product is not affected. The obtained values of these tests were found within normal limits which indicate good quality of product. The refractive index of *Kashmaryadi Ghrita* was found to be 1.4710, specific gravity was 0.9096, iodine value was 18,086, saponification value was 193.308 and acid value was 1.5990. HPTLC results showed 7 spots at 254 nm and 1 spot at 366 nm.

CONCLUSION

Pharmacognostical and physico-chemical evaluation of *Kashmaryadi Ghrita* illustrated the specific characters of ingredients which were used in the preparation. Physico-chemical analysis is an essential parameter for quality assurance which were found within prescribed limits in the present work. On the basis of observations and experimental results, this study may be used as a reference standard for further research work and clinical studies.

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