WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 8, 1066-1077. Research Article ISSN 2277–7105

PHARMACO ANALYTICAL STUDY OF KUTAJA KASHMARI GHRITHA

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Article Received on 15 March 2026,
Article Revised on 05 April 2026,
Article Published on 16 April 2026,

https://doi.org/10.5281/zenodo.19627816

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How to cite this Article: Dr. Preethi Hegde*[1], Dr. Mamatha K. V.[2], Dr. Anusha[3]. (2026). Pharmaco Analytical Study of Kutaja Kashmari Ghritha. World Journal of Pharmaceutical Research, 15(8), 1066–1077.

ABSTRACT

According to Ayurvedic Acharyas, the effectiveness of any formulation relies on its precise analysis and appropriate preparation. *Kutaja Kashmari Ghrita*, a traditional medicated ghee, is referenced in Ayurvedic texts for its utility in managing *Raktayoni*. This study involves the formulation and standardization of *Kutaja Kashmari Ghrita*, aiming to ensure quality, safety, and efficacy. The standardization process includes assessment of organoleptic properties, physicochemical parameters, and High-Performance Thin-Layer Chromatography (HPTLC) profiling, providing a scientific basis for its therapeutic application in *Asrigdhara*.

KEYWORDS: Abnormal Uterine Bleeding, Adenomyosis, Uterine Fibroids, Kutaja Kashmari Ghrita, Asrigdhara, HPTLC.

INTRODUCTION

Abnormal Uterine Bleeding (AUB), a key gynecological disorder, encompasses various etiologies under the PALM-COEIN classification system. Structural causes such as adenomyosis and uterine fibroids (leiomyomas) are frequent, leading to menorrhagia, pelvic pain, and reduced quality of life.[1] Conventional management often relies on hormonal

therapy or surgical intervention, which may not give permanent relief to the patients.

Charaka Samhita has explained *asrighdheeryate* ie excessive excretion of *Raja* in the context of defining *Asrigdhara.*[2] *Asrigdhara* is caused when *vayu* withholding the vitiated *rakta* reaches *rajovaha siras* of *garbhashaya* and then it increases the quantity of *rajas*. Hence the general treatment principles are related to alleviation of *vata* and *chikitsa* of *raktayoni, raktarshas, raktapitta, and rakta atisara.*[3]

MATERIALS AND METHODS

Collection, Identification and authentication of Raw drugs

All the raw material used for this study were procured from local market of Udupi Karnataka then Identification and authentication of the raw drug were Done at SDM Ayurveda pharmacy, GMP certified Pharmacy.

Method of preparation of *Kutaja kashmari ghritha*

Kutaja and *Kashmari* both are taken in equal quantity and *ghritha* is prepared by classical method of *ghritha* preparation until *sneha siddhi lakshanas* are attained.

Pharmacological Basis of *Kutaja Kashmari Ghrita*

Kutaja (Holarrhena antidysenterica)[4]

- *Rasa*: *Tikta* (bitter), *Kashaya* (astringent), *Katu* (pungent)
- *Guna*: *Ruksha* (dry), *Laghu* (light)
- *Veerya*: *Sheeta* (cold)
- *Vipaka*: *Katu* (pungent)
- *Doshagnata*: Pacifies *Pitta* and *Kapha*, also reduces *Rakta dushti*
- *Karma*:
- *Raktastambhaka* – arrests bleeding due to astringent and cooling properties
- *Sangrahi* – absorbs excess fluid from tissues and reduces discharge
- *Krimighna* – combats microbial load, which may contribute to inflammation
- *Deepana* – promotes digestive strength, essential for *dhatu pachana* (tissue metabolism)
- *Raktapittahara* – alleviates bleeding disorders

Kutaja's Kashaya-Tikta Rasa and *Sheeta Veerya* make it ideal for controlling excessive uterine bleeding, inflammation, and microbial aggravation seen in conditions like adenomyosis and fibroids.

***Kashmari (Gmelina arborea)*[5]**

- *Rasa*: *Madhura* (sweet), *Tikta* (bitter), *Kashaya* (astringent)
- *Guna*: *Guru* (heavy), *Snigdha* (unctuous)
- *Veerya*: *Sheeta* (cold)
- *Vipaka*: *Madhura* (sweet)
- *Doshagnata*: Pacifies *Pitta* and *Vata doshas*
- *Karma*:
- *Raktapittahara* – controls bleeding caused by aggravated *Pitta* and *Rakta*
- *Shothahara* – reduces inflammatory swelling in uterine tissues
- *Brihmana* – nourishes depleted tissues and improves endometrial integrity
- *Dahaprashamana* – alleviates burning sensations and Pitta-related inflammation
- *Garbhasthapana* – promotes uterine health and stabilizes reproductive functions

The *Madhura* and *Sheeta* qualities of *Kashmari* help to soothe inflamed endometrial linings, crucial in reversing the chronic pathology of AUB.

Together, *Kutaja* and *Kashmari* provide a synergistic *Ayurvedic* approach targeting the *samprapti* of *Asrigdhara*– reducing *Rakta srava*, balancing *Pitta-Vata*, and restoring *Yoni sthiti* (uterine integrity).

Role of *Ghrita*

Enhances potency preservation and supports tissue regeneration.

ANALYTICAL STUDY METHODOLOGY[6]

Refractive index

Placed a drop of water on the prism and adjusted the drive knob in such a way that the boundry line intersects the separatrix exactly at the centre. Noted the reading. Distilled water has a refractive index of 1.33182 at 31˚C. The difference between the reading and 1.3315gives the error of the instrument. If the reading is less than 1.33182, the error is minus (-) then the correction is plus (+) if the reading is more, the error is plus (+) and the correction is minus (-). Refractive index of oil is determined using 1 drop of the sample. The correction if any should be applied to the measured reading to get the accurate refractive index. Refractive index of the test samples were measured at 35˚C.

Specific gravity

Cleaned a specific gravity bottle by shaking with acetone and then with ether. Dried the bottle

and noted the weight. Cooled the sample solution to room temperature. Carefully filled the specific gravity bottle with the test liquid, inserted the stopper and removed the surplus liquid. Noted the weight. Repeated the procedure using distilled water in place of sample solution.

Viscosity

The given sample is filled in a U tube viscometer in accordance with the expected viscosity of the liquid so that the fluid level stands within 0.2 mm of the filling mark of the viscometer when the capillary is vertical and the specified temperature is attained by the test liquid. The liquid is sucked or blown to the specified height of the viscometer and the time taken for the sample to pass the two marks is measured. Viscosity is measured using the formula

$$\eta 1 = \frac{\rho 1 t 1 \text{ X } \eta 2}{\rho 2 t 2}$$

η1 – Viscosity of sample

η2 - Viscosity of water

t1 and t 2- time taken for the sample and water to pass the meniscus

ρ1 and ρ2 – Density of sample and water

X= Specific gravity of sample x 0.9961/specific gravity of water

Π= X x Time for samplex1.004/specific gravity of waterx70sec

Determination of pH

Preparation of buffer solutions

Standard buffer solution: Dissolved one tablet of pH 4, 7 and 9.2 in 100 ml of distilled water.

Determination of pH: 1 ml of sample was taken and make up to 10 ml with distilled water, stirred well and filtered. The filtrate was used for the experiment. Instrument was switched on. 30 minutes time was given for warming pH meter. The pH 4 solution was first introduced and the pH adjusted by using the knob to 4.02 for room temperature 30°C. The pH 7 solution was introduced and the pH meter adjusted to 7 by using the knob. Introduced the pH 9.2 solution and checked the pH reading without adjusting the knob. Then the sample solution was introduced and reading was noted. Repeated the test four times and the average reading were taken as result.

Acid value

Weighed 2- 10g of *Kutaja kashmari ghritha* in a conical flask. Added 50 ml of acid free

alcohol-ether mixture (25 +25ml) previously neutralised with the 0.1M potassium hydroxide solution and shaken well. Added One ml of Phenolphthalein solution and titrated against 0.1M Potassium hydroxide solution. End point is the appearance of pale pink colour. Repeated the experiment twice to get concordant values.

Acid value = a (TV in ml) X 0.00561X1000/ W

Saponification value

Weighed 2g of the *Kutaja kashmari ghritha* into a 250 ml RB flask fitted with a reflux condenser. Added 25ml of 0.5M alcoholic potash. Refluxed on a water bath for 30 minutes. Cooled and added 1 ml of Phenolphthalein solution and titrated immediately with 0.5 M Hydrochloric acid (a ml). Repeated the operation omitting the substance being examined (blank) (b ml). Repeated the experiment twice to get concordant values.

Saponification value = (b-a) x 0.02805x1000/W

Iodine value

The sample *Kutaja kashmari ghritha* 0.1g was accurately weighed in a dry iodine flask. Dissolved with 10ml of CCl_4, 20ml of iodine monochloride solution was added. Stopper was inserted, which was previously moistened with solution of potassium iodide and flask was kept in a dark place at a temperature of about 17^0 C for 30 min. 15ml of potassium iodide and 100ml of water was added and shaken well. This was titrated with 0.1N Sodium thiosulphate, starch was used as indicator. The number of ml of 0.1N sodium thiosulphate required (a) was noted. The experiment was repeated with the same quantities of reagents in the same manner omitting the substance. The number of ml of 0.1N sodium thiosulphate required (b) was noted. The experiment was repeated twice to get concordant values.

Iodine value = (b-a) x 0.01269x100/W

Peroxide value

5g of the *Kutaja kashmari ghritha* was weighed accurately into a conical flask, added 30 ml of mixture of 3volumes of glacial acetic acid and 2 volumes of chloroform, added 0.5ml of potassium iodide, allowed it to stand for 1 minute, add 30ml of water titrate gradually with vigorous shaking with 0.1M sodium thiosulphate until the yellow color disappears. Add 0.5ml of starch indicator continued the titration until blue color disappears.

Peroxide value= 10(a-b)/W

Where W= weight in g of the substance

Rancidity test

1ml of melted fat was mixed with 1ml of conc. Hcl and 1ml of 1% solution of phloroglucinol in diethyl ether and then mixed thoroughly with the fat acid mixture. A pink color indicates that the fat is slightly oxidized while a red color indicates that the fat is definitely oxidized.

Determination of Unsaponifiable matter

Weighed 5g of the *Kutaja kashmari ghritha* into the flask. added 50ml alcoholic KOH into the sample. Boiled gently but steadly under reflux condenser for one hour. The condensor was washed with 10ml of ethyl alcohol and the mixture was collected and transferred to a separating funnel. The transfer was completed by washing the sample with ethyl alcohol and cold water. Altogether, 50ml of water was added to the separating funnel followed by an addition of 50ml petroleum ether. The stopper was inserted and shaken vigorously for 1 minute and allowed it to settle until both the layers were clear. The lower layer containing the soap solution was transferred to another separating funnel and repeated the ether extraction six times more using 50ml of petroleum ether for each extraction. All the extracts were collected in a separating funnel. The combined extracts were washed in the funnel 3 times with 25ml of aqueous alcohol and shaked vigorously. And drawing off the alcohol-water layer after each washing. The ether layer was again washed repeatedly with 25ml of water until the water no longer turns pink on addition of a few drops of Phenolphthalein indicator solution. The ether layer was transferred to a tarred flask containing few pieces of pumice stone and evaporated to dryness on a water bath. Placed the flask in an air oven at 85°c for about 1 hour to remove the last traces of ether. A few ml of acetone was added and evaporated to dryness on a water bath. Cooled in a desicator to remove last traces of moisture and then weighed.

Sample preparation for HPTLC

Sample obtained in the procedure for the determination of unsaponifiable matter is dissolved in 10 ml of chloroform this was followed for the sample of *Kutaja kashmari ghritha*, and chloroform soluble portion was used for TLC.

HPTLC

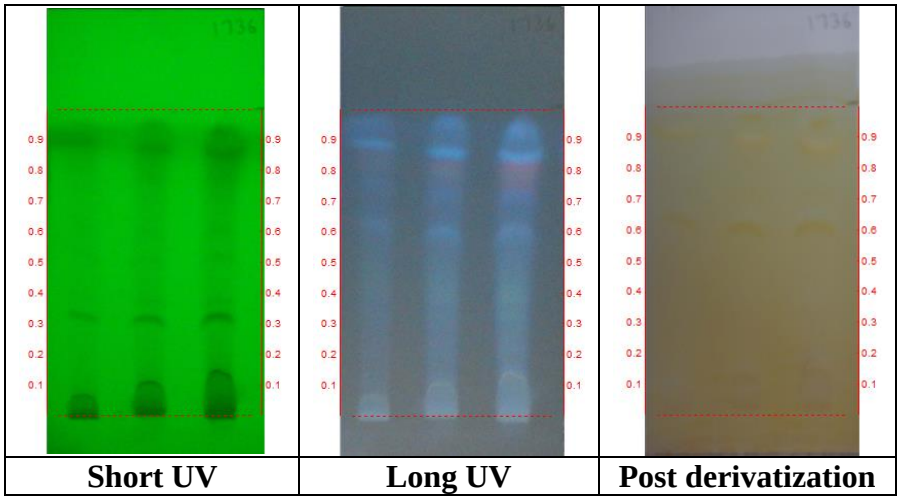
3, 6, 9µl of the chloroform fraction of samples of *Kutaja kashmari ghritha* was applied on a precoated silica gel F_{254} on aluminum plates to a band width of 8mm using Linomat 5 TLC applicator. The plate was developed in Cyclohexane: Chloroform: Diethylamine (7:2:1) and the developed plates were visualized under short UV, long UV and after derivatisation with

dragendroffs spray reagent and scanned under UV 254nm, 366nm, 620nm (after derivatisation). Rf, color of the spots and densitometric scan were recorded.

RESULTS

Table 1: Results of standardization parameters for *Kutaja kashmari ghritha.*

Parameter	***Results n = 3 %w/w***
Color	Yellow
Odour	Pleasant, Characteristic
Refractive index	1.45832
Specific gravity	0.891
Viscosity	-
pH	6.0
Acid value	4.95
Saponification value	264.84
Iodine Value	8.96-12.69
Peroxide value	0.19
Unsaponifiable matter (%)	1.90
Rancidity	Fat is not oxidized



Short UV	**Long UV**	**Post derivatization**

Track 1 - *Kutaja kashmari ghritha* – 3µl

Track 2 - *Kutaja kashmari ghritha* – 6µl

Track 3 - *Kutaja kashmari ghritha* – 9µl

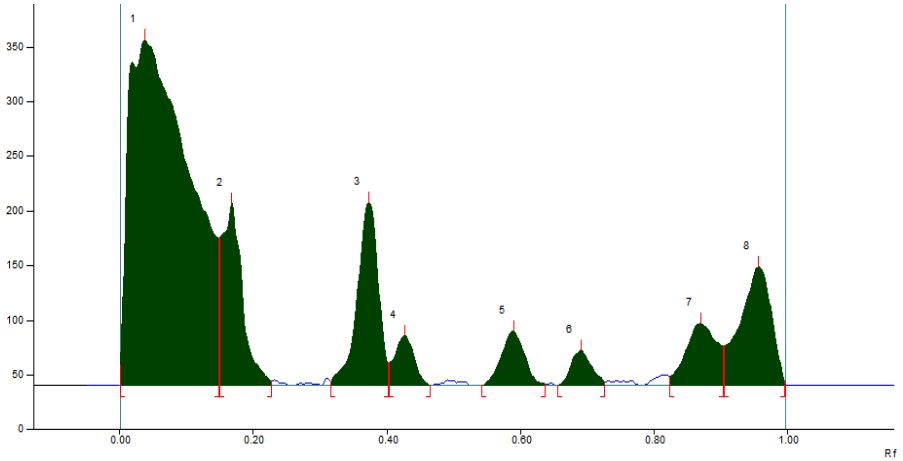
Solvent system – Cyclohexane: Chloroform: Diethylamine (7:2:1), R_f -Conessine (0.82)

Figure 1: HPTLC photo documentation of Chloroform fraction of Kutaja kashmari ghritha.

Table 1: R_f values of sample of *Kutaja kashmari ghritha.*

Short UV	Long UV	Post derivatization
0.11 (D. green)	0.13 (f. blue)	0.12 (Yellow)
0.32 (D. green)	0.32 (f. blue)	-
0.38 (D. green)	0.40 (f. blue)	-
0.53 (D. green)	-	-
-	0.60 (f. blue)	0.62 (Yellow)
0.76 (D. green)	0.74 (f. blue)	-
-	0.81 (f. red)	-
0.87 (D. green)	0.86 (f. blue)	0.87 (Yellow)
-	0.93 (f. blue)	-

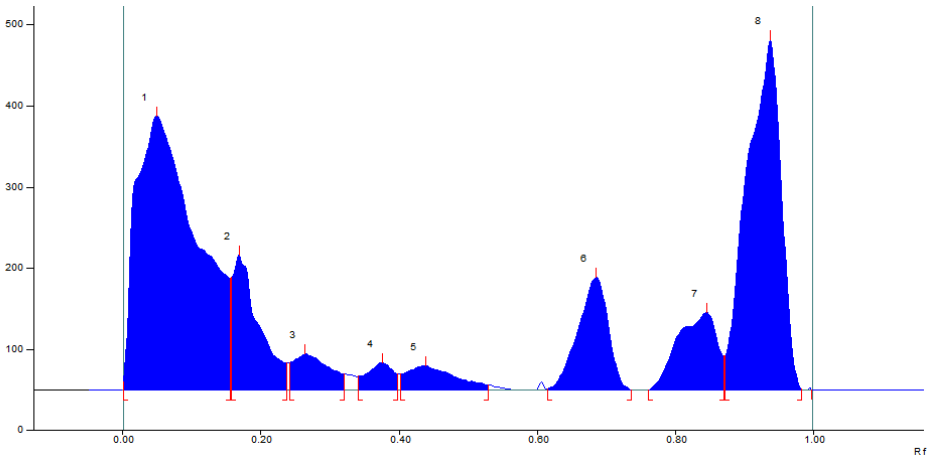
***F – Fluorescent; L –Light; D – Dark**



Track 3, ID: Kutaja kashmari ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	18.1 AU	0.04 Rf	315.7 AU	33.54 %	0.15 Rf	34.4 AU	20818.2 AU	56.81 %
2	0.15 Rf	134.8 AU	0.17 Rf	166.7 AU	17.71 %	0.23 Rf	3.8 AU	3666.4 AU	10.01 %
3	0.32 Rf	3.6 AU	0.37 Rf	167.1 AU	17.75 %	0.40 Rf	20.5 AU	3780.8 AU	10.32 %
4	0.40 Rf	21.1 AU	0.43 Rf	45.6 AU	4.84 %	0.47 Rf	0.0 AU	941.2 AU	2.57 %
5	0.54 Rf	0.0 AU	0.59 Rf	49.5 AU	5.26 %	0.64 Rf	1.5 AU	1231.7 AU	3.36 %
6	0.66 Rf	0.2 AU	0.69 Rf	32.3 AU	3.43 %	0.73 Rf	3.3 AU	704.4 AU	1.92 %
7	0.82 Rf	8.2 AU	0.87 Rf	56.4 AU	5.99 %	0.90 Rf	35.8 AU	1880.7 AU	5.13 %
8	0.91 Rf	36.1 AU	0.96 Rf	108.0 AU	11.47 %	1.00 Rf	3.2 AU	3620.7 AU	9.88 %

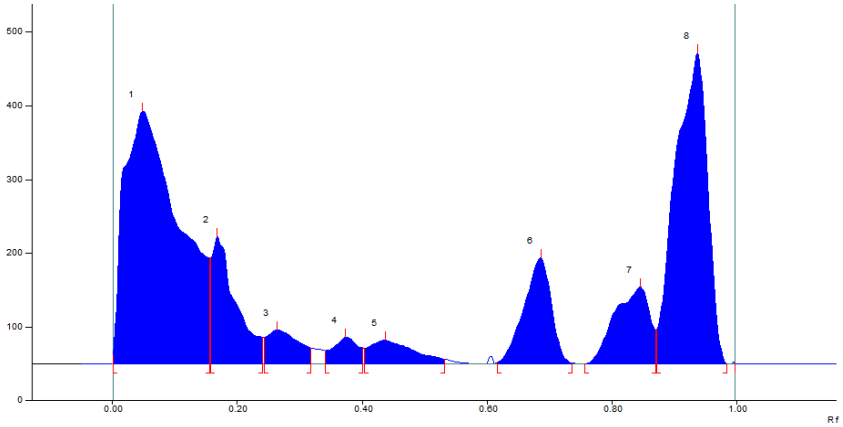
Fig. 2a: At 254nm. R_f -Conessine (0.82).



Track 3, ID: Kutaja kashmari ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	10.5 AU	0.05 Rf	337.7 AU	26.43 %	0.16 Rf	38.0 AU	21497.7 AU	40.11 %
2	0.16 Rf	139.2 AU	0.17 Rf	166.6 AU	13.04 %	0.24 Rf	33.8 AU	4515.1 AU	8.42 %
3	0.24 Rf	34.0 AU	0.26 Rf	44.3 AU	3.47 %	0.32 Rf	19.6 AU	1673.4 AU	3.12 %
4	0.34 Rf	16.8 AU	0.38 Rf	33.3 AU	2.60 %	0.40 Rf	20.0 AU	908.3 AU	1.69 %
5	0.40 Rf	19.8 AU	0.44 Rf	30.1 AU	2.35 %	0.53 Rf	6.3 AU	1508.0 AU	2.81 %
6	0.62 Rf	1.4 AU	0.69 Rf	139.0 AU	10.88 %	0.74 Rf	0.1 AU	4330.6 AU	8.08 %
7	0.76 Rf	0.1 AU	0.85 Rf	95.4 AU	7.46 %	0.87 Rf	41.7 AU	3893.4 AU	7.26 %
8	0.87 Rf	42.1 AU	0.94 Rf	431.5 AU	33.77 %	0.98 Rf	0.7 AU	15267.1 AU	28.49 %

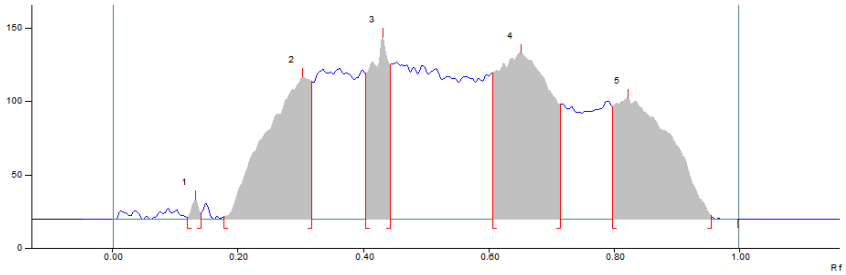
Fig. 2b: At 366nm, Hg, flu, R_f -Conessine 0.82±0.03.



Track 3, ID: Kutaja kashmari ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	11.4 AU	0.05 Rf	342.6 AU	26.38 %	0.16 Rf	43.3 AU	21929.0 AU	39.66 %
2	0.16 Rf	144.3 AU	0.17 Rf	172.4 AU	13.27 %	0.24 Rf	36.4 AU	4785.0 AU	8.65 %
3	0.24 Rf	36.3 AU	0.26 Rf	46.5 AU	3.58 %	0.32 Rf	21.4 AU	1697.4 AU	3.07 %
4	0.34 Rf	17.9 AU	0.37 Rf	36.3 AU	2.80 %	0.40 Rf	21.8 AU	1024.4 AU	1.85 %
5	0.40 Rf	21.4 AU	0.44 Rf	32.0 AU	2.46 %	0.53 Rf	6.1 AU	1635.6 AU	2.96 %
6	0.62 Rf	2.1 AU	0.69 Rf	143.7 AU	11.06 %	0.74 Rf	1.3 AU	4508.3 AU	8.15 %
7	0.76 Rf	0.1 AU	0.85 Rf	104.1 AU	8.01 %	0.87 Rf	46.0 AU	4175.8 AU	7.55 %
8	0.87 Rf	46.5 AU	0.94 Rf	421.4 AU	32.44 %	0.99 Rf	0.1 AU	15540.9 AU	28.10 %

Fig. 2c: At 366nm, Hg,Abs, R_f -Conessine 0.82±0.03.



Track 3, ID: Kutaja kashmari ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.12 Rf	1.2 AU	0.13 Rf	13.6 AU	3.17 %	0.14 Rf	3.7 AU	105.1 AU	0.53 %
2	0.18 Rf	1.8 AU	0.30 Rf	96.5 AU	22.47 %	0.32 Rf	93.6 AU	4932.8 AU	24.75 %
3	0.40 Rf	99.1 AU	0.43 Rf	124.2 AU	28.90 %	0.44 Rf	05.0 AU	2703.7 AU	13.56 %
4	0.61 Rf	99.4 AU	0.65 Rf	112.6 AU	26.21 %	0.71 Rf	77.8 AU	6819.5 AU	34.21 %
5	0.80 Rf	76.3 AU	0.82 Rf	82.7 AU	19.25 %	0.96 Rf	2.4 AU	5373.0 AU	26.95 %

Fig. 2d: At 620nm (after derivatisation with Dragendroffs reagent), R_f -Conessine 0.82.

Figure 2: Densitometric scan of *Kutaja kashmari ghritha.*

DISCUSSION[7]

The management of Abnormal Uterine Bleeding (AUB) due to adenomyosis and uterine fibroids requires addressing excessive endometrial shedding, chronic inflammation, myometrial hypertrophy, and vascular congestion.

1. *Kutaja (Holarrhena antidysenterica)*

Major Phytoconstituents and Their Actions

Phytoconstituent	Chemical Nature	Pharmacological Action	Relevance in AUB/Asrigdara
Conessine	Alkaloid	Anti-inflammatory, anti-amoebic, astringent	Controls inflammation, reduces vascular permeability and excessive bleeding
Holarrhenine	Alkaloid	Hemostatic, uterotonic	Promotes vasoconstriction and helps reduce uterine bleeding
Holarrhidine	Alkaloid	Antioxidant, antimicrobial	Prevents microbial-induced uterine irritation and bleeding
Tannins	Polyphenolic	Astringent, vasoconstrictive	Constricts capillaries and reduces capillary fragility in uterine vessels
Steroidal alkaloids	Steroid-like compounds	Anti-inflammatory, hormonal modulator	Supports hormonal regulation and reduces endometrial congestion
Resins	Mixed phytochemicals	Protective, astringent	Coats and protects uterine lining, prevents irritation

Mechanism of Action in AUB

- Conessine and other alkaloids stabilize blood vessels and reduce capillary leakage.
- Tannins have a vasoconstrictive effect that helps to reduce blood flow in hyperemic uterine tissues.
- The anti-inflammatory action aids in calming the endometrial inflammation, which is often a contributing factor in heavy bleeding.
- Antimicrobial properties protect from secondary infections which may exacerbate menstrual bleeding.

2. *Kashmari (Gmelina arborea)*

Major Phytoconstituents and Their Actions

Phytoconstituent	Chemical Nature	Pharmacological Action	Relevance in AUB/Asrigdara
Gmelinol	Lignan	Phytoestrogenic, antioxidant	Interact with estrogenic pathways
Flavonoids (quercetin, kaempferol)	Polyphenolic antioxidants	Anti-inflammatory, capillary stabilizer	Protects endometrial vessels from damage and inflammation
Phytosterols	Plant sterols	Hormone-modulating, regenerative	Promotes repair of damaged endometrial lining
Tannins	Polyphenolic	Astringent, antihemorrhagic	Constricts blood vessels and reduces blood loss
Saponins	Glycosides	Anti-inflammatory, immunomodulatory	Reduces inflammation and uterine irritation

Mechanism of Action in AUB

- Lignans act as natural phytoestrogens, helping to regulate estrogen-progesterone balance.
- Flavonoids and sterols help repair damaged endometrial vessels and reduce abnormal proliferation.
- Tannins and saponins have astringent and anti-inflammatory properties that reduce bleeding and pain.
- These compounds also exhibit tonic effects on the reproductive system.

CONCLUSION

Kutaja kashmari ghritha addresses the triad of pathology in AUB—bleeding, inflammation, and tissue degeneration—through its classical pharmacological profile and scientifically validated composition. Hence it is a classically sound, pharmacologically potent, and analytically validated *Ayurvedic* formulation.

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