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# PHYSICOCHEMICAL, PHYTO CHEMICAL AND HPTLC EVALUATION OF SHILODBIDADI TAILA AND GOKSHURA KWATHA CHURNA

**Dr. Veeresh Sattigeri [1] , Dr.Siddanagouda A Patil [2] ,**

[1] PhD Scholar, Shalyatantra Dept., Ayurved Mahavidyalaya, Hubli, Karnataka, India &

HOD and Reader Dept of Shalyatantra Shri Veer Pulikeshi Rural Ayurvedic Medical college Badami.

[2]Associate Professor, Shalyatantra Dept, Ayurved Mahavidyalaya, Hubli, Karnataka, India

## Abstract

B.P.H. is condition related to ageing process and most frequently seen in men in 7th, 8th, 9th decade, but also occurs in 6th and even 5th decade of life. Surveys have found a high prevalence of moderate to severe obstructive symptom in men over 50, which increases with age. increase in BenignProstate Hypertrophy and lower urinary tract symptoms such as urgency, dribbling micturition, hesitancy, and increase frequency of micturition are on rise, occurring within the context of an aging global population. In Ayurvedic classics the term Mutraghata is related with the symptoms of low urinary output either by retention, absolute or relative anuria or oliguria. In relation to BPH condition, clinical study was conducted in AMV Hubli with an objective to assess the efficacy of shilodbidadi taila matra basti and Gokshuradi kwatha and in management of .*0* w.s.r to BPH.

To highlight its mode of action accurately the Pharmacological analysis and HPTLC Study on the same helps in proper understanding and interpretation of drug action. This article enlightens about the Pharmacological analysis and HPTLC Study of shilodbidadi taila and Gokshuradi kwatha.

As per the Analysis reports The standardisation of Shilodbidadi taila and Gokshura kwatha churna was performed and results of which are 1. Phytochemical test performed qualitatively shows presence of alkaloids, glycosides, steroids, taanins, flavonoids, saponins, terpenoids, phenols and resins. HPTLC was performed for *Shilodbidadi taila* and *Gokshura kwatha churna.*

**KEYWORDS:** Physicochemical, Phytochemicals, *Shilodbidadi taila* and *Gokshura kwatha churna.*

## Introduction

BPH is a common and progressive disease of aging men According to *Ayurveda* classics *Mutraghata* is a diseased condition where 'retention of urine' is the cardinal feature, whereas in *Mutrakricchara*, difficulty in micturition is the typical characteristic feature. Obstruction and hence retention of urine may be caused by occlusion of the urinary tract or inflammation in the urinary pathway. Often injury, constriction/compressed stones or any other possible foreign bodies may result in this pathological condition. So it is the need of the hour to understand BPH in terms of *Ayurveda* so that an uncomplicated and patient friendly treatment can be advised to the patients. Keeping all these facts in mind a clinical study "A Clinical Study with *shilodbidadi taila matra basti* and *Gokshuradi kwatha* in the Management of *Mutraghata* w.s.r. to BPH" was designed. *Shilodbidadi taila matra basti* and *Gokshuradi kwatha* seems to be appropriate drugs according to *Ayurvedic* classics in relation to the management of *Mutraghata* .

To highlight its mode of action accurately the Pharmacological analysis and HPTLC Study on the same helps in proper understanding and interpretation the drug action. This article enlightens about the Pharmacological analysis and HPTLC Study of *Shilodbidadi taila* and *Gokshuradi kwatha*

**Investigation to be performed:** Refractive index, specific gravity, viscosity, Acid value, Saponification value, Iodine value, Peroxide value, HPTLC

Sample details: *Shilodbidadi taila* and *Gokshura kwatha churna*

**Methodology**

**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 1.33217 at 28˚C. The difference between the reading and1.3320 gives the error of the instrument. If the

reading is less than 1.3320, 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 28˚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

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

$\eta 1$ – Viscosity of sample

$\eta 2$ - Viscosity of water

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

$\rho 1$ and $\rho 2$ – Density of sample and water

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

$\Pi$= X x Time for samplex1.004/specific gravity of waterx70sec

**Acid value**

Weighed 2- 10g of Shilodbidadi taila 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.

**Saponification value**

Weighed 2g of the Shilodbidadi taila 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.

**Iodine value**

About 0.1g of the Shilodbidadi taila 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.

**Determination of Unsaponifiable matter**

Weighed 5g of the Shilodbidadi taila 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 all the sample of Shilodbidadi taila, and chloroform soluble portion was used for HPTLC.

**HPTLC:**

3, 6, 9µl of the chloroform fraction of samples of Shilodbidadi taila was applied on a precoated silica gel F254 on aluminum plates to a band width of 8 mm using Linomat 5 TLC applicator. The plate was developed in Toluene – Ethyl acetate (9:1) and the developed plates were visualized under short UV, long UV and after derivatisation in vanillin-sulphuric acid spray reagent and scanned under UV 254nm, 366 nm and 620nm (Post derivatisation). Rf, color of the spots and densitometric scan were recorded.

**Peroxide value**

5g of the Shilodbidadi taila 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

**Loss on drying at 105ºC**

10 g of Gokshura kwatha churna was placed in tared evaporating dish. It was dried at 105˚C for 5 hours in hot air oven and weighed. The drying was continued until difference between two successive weights was not more than 0.01 after cooling in desiccator. Percentage of moisture was calculated with reference to weight of the sample.

**Total Ash**

2 g of Gokshura kwatha churna was incinerated in a tared platinum crucible at temperature not exceeding 450˚C until carbon free ash is obtained. Percentage of ash was calculated with reference to weight of the sample.

**Acid insoluble Ash**

To the crucible containing total ash, add 25ml of dilute HCl and boil. Collect the insoluble matter on ashless filter paper (Whatmann 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccator for 30 mins and weigh without delay. Calculate the content of acid insoluble ash with reference to the air dried drug.

**Alcohol soluble extractive**

Weigh accurately 4 g of the Gokshura kwatha churna in a glass stoppered flask. Add 100 ml of distilled Alcohol (approximately 95%). Shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours, cool in desiccator for 30 minutes and weigh. Calculate the percentage of Alcohol extractable matter of the sample. Repeat the experiment twice, and take the average value.

**Water soluble extractive**:

Weigh accurately 4 g of the Gokshura kwatha churna in a glass stoppered flask. Add 100 ml of distilled water, shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours. Cool in a desiccator and weigh. Repeat the experiment twice. Take the average value.

**Preliminary phytochemical tests**

**Tests for alkaloids *Dragendroff's test:*** To a few mg of extract dissolved in alcohol, a few drops of acetic acid and Dragendroff's reagent were added and shaken well. An orange red precipitate formed indicates the presence of alkaloids.

- ***Wagners's test:*** To a few mg of extract dissolved in acetic acid, a few drops of Wagner's reagent was added. A reddish brown precipitate formed indicates the presence of alkaloids.
- ***Mayer's test:*** To a few mg of extract dissolved in acetic acid, a few drops of Mayer's reagent was added. A dull white precipitate formed indicates the presence of alkaloids.
- ***Hager's test:*** To a few mg of extract dissolved in acetic acid, 3 ml of Hager's reagent was added, the formation of yellow precipitate indicates the presence of alkaloids.

**Tests for carbohydrates**

- ***Molisch's test:*** To the extract, 1 ml of $\alpha$-naphthol solution and conc. sulphuric acid were added along the sides of test tube. Violet colour formed at the junction of the two liquids indicates the presence of carbohydrates.
- ***Fehling's test:*** A few mg of extract was mixed with equal quantities of Fehling's solution A and B. The mixture was warmed on a water bath. The formation of a brick red precipitate indicates the presence of carbohydrates.
- ***Benedict's test:*** To 5 ml of Benedict's reagent, a few mg of extract was added, and boiled for two minutes and cooled. Formation of a red precipitate indicates the presence of carbohydrates.

**Test for steroids**

- ***Libermann-Burchard test:*** To the extract was dissolved in chloroform, 1 ml of acetic acid and 1 ml of acetic anhydride were added, then heated on a water bath and cooled. Few drops of conc. Sulphuric acid were added along the sides of the test tube. Appearance of bluish green colour indicates the presence of steroids.

- ***Salkowski test:*** The extract was dissolved in chloroform and equal volume of conc. Sulphuric acid was added. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer indicates the presence of steroids.

**Test for saponins**

To a few mg of extract, distilled water was added and shaken. Stable froth formation indicates the presence of saponin.

**Test for tannins**

To the extract, a few drops of dilute solution of ferric chloride was added, formation of dark blue colour shows the presence of tannins.

**Test for flavonoids**

***Shinoda's test:*** To the extract in alcohol, a few magnesium turnings and few drops of conc. hydrochloric acid were added and heated on a water bath. Formation of red to pink colour indicates the presence of flavonoids.

**Test for phenol**

To the extract in alcohol, added two drops of alcoholic ferric chloride. Formation of blue to blue black indicates the presence of phenol.

**Test for coumarins**

To the extract in alcohol, a few drops of 2 N sodium hydroxide solution was added. Dark yellow colour formation indicates the presence of coumarins.

**Test for triterpenoids**

The extract was warmed with tin bits and few drops of thionyl chloride. Formation of pink colour indicates the presence of triterpenoids.

**Test for carboxylic acid**

Extract dissolved in water is treated with sodium bicarbonate. Brisk effervescence indicates the presence of carboxylic acid.

**Test for resin**

Few mg of the sample was mixed with water and acetone. Turbidity indicates the presence of resins.

**Test for quinone**

A few mg of alcohol extract was treated with 0.5% of sodium hydroxide. Deep coloration like pink, purple or red indicates the presence of quinone.

**HPTLC**

1g of *Gokshura kwatha* churna powder was extracted with 10 ml of alcohol . 3, 6 and 9µl of the above extract was applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (8.0: 1.0). The developed plates were visualized in short UV, long UV and then derivatised with vanillin sulphuric acid and scanned under 254nm, 366nm and 620nm. Rf, colour of the spots and densitometric scan were recorded.

## Part C: Results

**Table 1: Standardization parameter of Shilodbidadi taila and Gokshura kwatha churna**

| Parameter | *Results n = 3 %w/w* | |
|---|---|---|
| | **Shilodbidadi taila** | **Gokshura kwatha churna** |
| Refractive index | 1.4684 | - |
| Specific gravity | 0.9153 | - |
| Viscosity | 58.27 | - |
| Acid value | 1.09 | - |
| Saponification value | 278.82 | - |
| Iodine value | 68.92 | - |
| Unsaponifiable matter (%) | 2.78 | - |
| Peroxide value | 0.0 | - |
| Loss on drying | - | 7.64±0.01 |
| Total Ash | - | 16.08±0.01 |
| Acid Insoluble Ash | - | 4.28±0.00 |
| Water soluble Ash | - | 1.8±0.00 |
| Alcohol soluble extractive value | - | 7.69±0.00 |
| Water soluble extractive value | - | 20.78±0.02 |

**Table 2: Results of preliminary phytochemical screening of Gokshura kwatha churna**

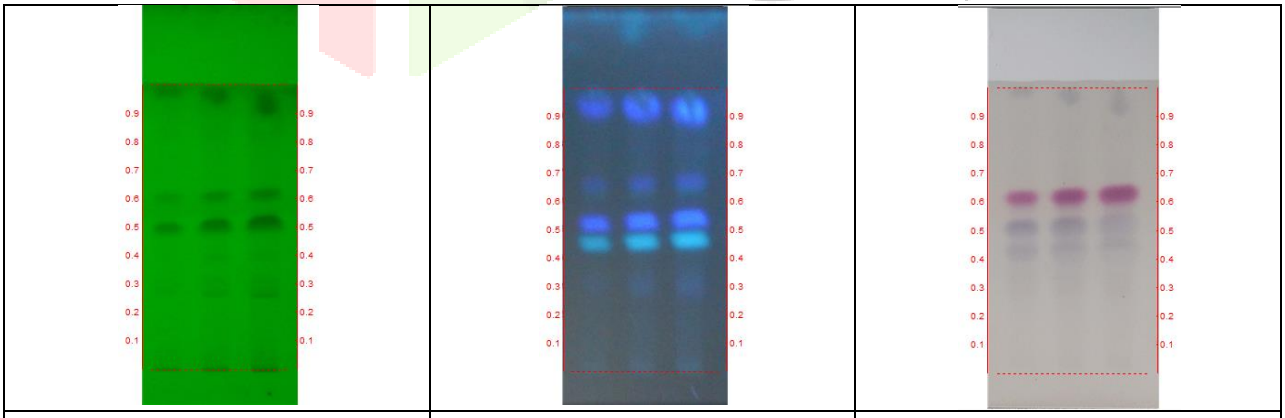
| Test | Inference |
|---|---|
| Alkaloid | + |
| Steroid | + |
| Carbohydrate | + |
| Tannin | + |
| Flavanoids | + |
| Saponins | + |
| Terpenoid | + |
| Coumarins | - |
| Phenols | + |
| Carboxylic acid | - |
| Amino acids | - |
| Resin | + |
| Quinone | - |

(+) - present; (-) – negative

| **Tests** | **Color if positive** | Alcoholic extract *Gokshura kwatha churna* |
|---|---|---|
| **Alkaloids** | | |
| Dragendroff's test | Orange red precipitate | Orange red precipitate |
| Wagners test | Reddish brown precipitate | Reddish brown precipitate |
| Mayers test | Dull white precipitate | Dull white precipitate |
| Hagers test | Yellow precipitate | Yellow precipitate |
| **Steroids** | | |
| Liebermann-buchard test | Bluish green colour | Bluish green colour |
| Salkowski test | Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer | Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer |
| **Carbohydrate** | | |
| Molish test | Violet ring | Violet ring |
| Fehlings test | Brick red precipitate | Brick red precipitate |
| Benedicts test | Red precipitate | Red precipitate |
| **Tannin** | | |
| With $FeCl_3$ | Dark blue or green or brown | Brown color |
| **Flavanoids** | | |
| Shinoda's test | Red or pink | Pink color |

| | | |
|---|---|---|
| **Saponins** | | |
| With $NaHCO_3$ | Stable froth | Stable froth |
| **Triterpenoids** | | |
| Tin and thionyl chloride test | Pink | Pink |
| **Coumarins** | | |
| With 2 N NaOH | Yellow | Brown color |
| **Phenols** | | |
| With alcoholic ferric chloride | Blue to blue black | Bluish black color |
| **Carboxylic acid** | | |
| With water and $NaHCO_3$ | Brisk effervescence | No brisk effervescence |
| **Amino acid** | | |
| With ninhydrine reagent | Purple colour | No purple color |
| **Resin** | | |
| With aqueous acetone | Turbidity | Turbidity |
| **Quinone** | | |
| Conc. sulphuric acid | Pink/purple/red | Greenish brown color |

**Figure 1. HPTLC photo documentation of chloroform fraction of *Shilodbidadi taila***



| Short UV | Long UV | Under white light (after derivatisation) |
|---|---|---|

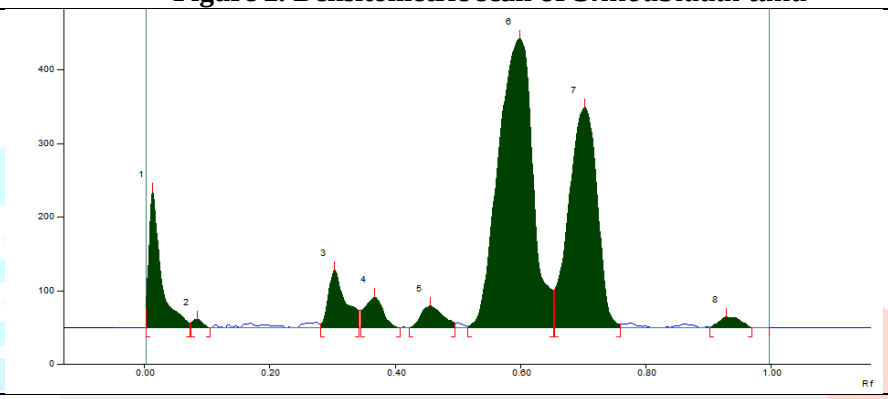
Track 1- ***Shilodbidadi taila***– 3µl
Track 2- ***Shilodbidadi taila***– 6µl
Track 3- ***Shilodbidadi taila***– 9µl

**Solvent system – Toluene: Ethyl Acetate (9:1)**

**Table 3 : Rf value of Shilodbidadi taila**

| Short UV | Long UV | Under white light (after derivatisation) |
|---|---|---|
| 0.26 (Green) | - | - |
| - | 0.30 (F. blue) | 0.30 (Purple) |
| 0.32 (Green) | - | - |
| - | - | 0.35 (Purple) |
| 0.40 (Green) | - | - |
| - | - | 0.43 (Purple) |
| - | 0.46 (F aqua blue) | - |
| 0.51 (D. green) | - | 0.52 (Purple) |
| - | 0.54 (F. blue) | - |
| 0.62 (D. green) | - | 0.63 (Pink) |
| - | 0.67 (F. blue) | - |
| 0.84 (Green) | - | - |

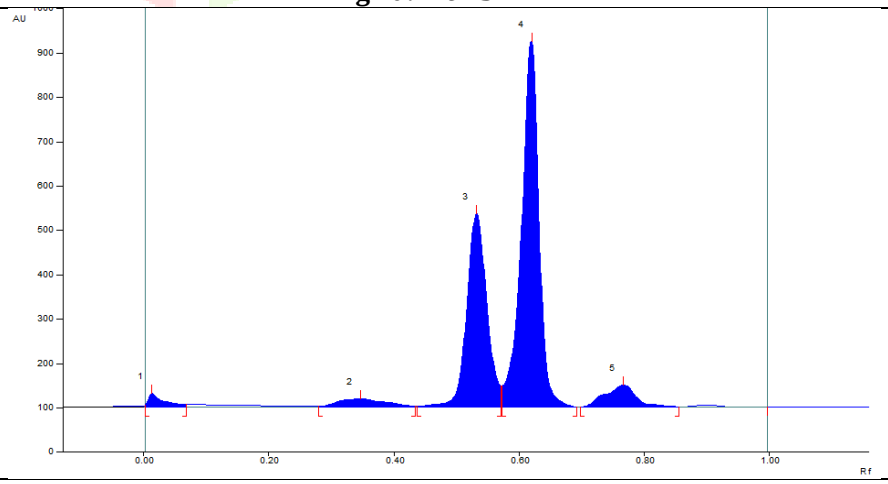
**Figure 2. Densitometric scan of *Shilodbidadi taila***




Track 7, ID: Shilodbidadi taila

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.00 Rf | 25.5 AU | 0.01 Rf | 184.5 AU | 17.56 % | 0.07 Rf | 6.7 AU | 2539.4 AU | 8.12 % |
| 2 | 0.07 Rf | 7.1 AU | 0.08 Rf | 11.7 AU | 1.11 % | 0.10 Rf | 0.1 AU | 137.1 AU | 0.44 % |
| 3 | 0.28 Rf | 4.5 AU | 0.30 Rf | 77.9 AU | 7.41 % | 0.34 Rf | 23.3 AU | 1545.4 AU | 4.94 % |
| 4 | 0.35 Rf | 23.4 AU | 0.37 Rf | 41.7 AU | 3.96 % | 0.41 Rf | 0.2 AU | 871.5 AU | 2.79 % |
| 5 | 0.42 Rf | 0.0 AU | 0.46 Rf | 29.4 AU | 2.80 % | 0.50 Rf | 6.5 AU | 739.9 AU | 2.37 % |
| 6 | 0.52 Rf | 1.8 AU | 0.60 Rf | 392.1 AU | 37.33 % | 0.65 Rf | 51.0 AU | 15404.2 AU | 49.28 % |
| 7 | 0.65 Rf | 51.6 AU | 0.70 Rf | 299.0 AU | 28.46 % | 0.76 Rf | 4.4 AU | 9668.0 AU | 30.93 % |
| 8 | 0.90 Rf | 0.6 AU | 0.93 Rf | 14.3 AU | 1.36 % | 0.97 Rf | 0.0 AU | 354.7 AU | 1.13 % |

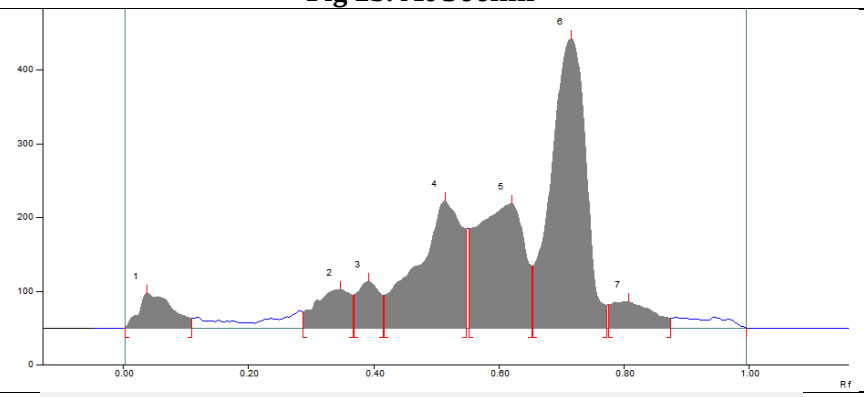
**Fig 2a. At 254nm**



Track 7, ID: Shilodbidadi taila

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.00 Rf | 3.4 AU | 0.01 Rf | 30.9 AU | 2.27 % | 0.07 Rf | 6.3 AU | 563.9 AU | 1.87 % |
| 2 | 0.28 Rf | 1.5 AU | 0.35 Rf | 19.1 AU | 1.41 % | 0.44 Rf | 2.1 AU | 1034.2 AU | 3.43 % |
| 3 | 0.44 Rf | 2.1 AU | 0.53 Rf | 436.6 AU | 32.10 % | 0.57 Rf | 48.3 AU | 10114.1 AU | 33.57 % |
| 4 | 0.57 Rf | 48.4 AU | 0.62 Rf | 823.5 AU | 60.55 % | 0.69 Rf | 0.1 AU | 16628.2 AU | 55.19 % |
| 5 | 0.70 Rf | 0.0 AU | 0.77 Rf | 50.0 AU | 3.67 % | 0.86 Rf | 0.8 AU | 1790.8 AU | 5.94 % |

**Fig 2b. At 366nm**

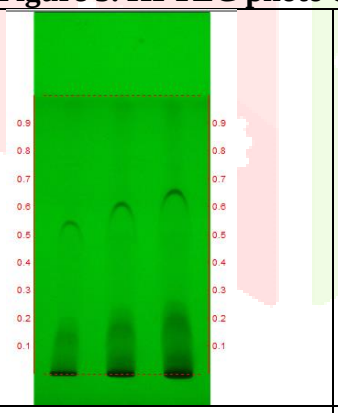



Track 7, ID: Shilodbidadi taila

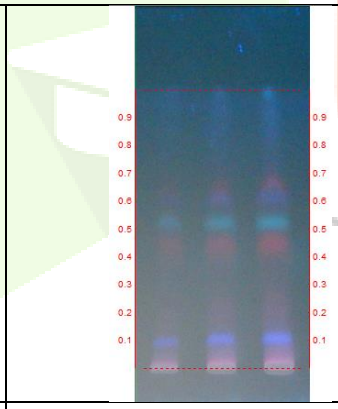
| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.00 Rf | 1.0 AU | 0.04 Rf | 47.4 AU | 5.09 % | 0.11 Rf | 13.4 AU | 1832.8 AU | 4.51 % |
| 2 | 0.29 Rf | 22.5 AU | 0.35 Rf | 52.3 AU | 5.61 % | 0.37 Rf | 44.8 AU | 2114.1 AU | 5.20 % |
| 3 | 0.37 Rf | 45.3 AU | 0.39 Rf | 63.2 AU | 6.78 % | 0.42 Rf | 44.6 AU | 1631.3 AU | 4.01 % |
| 4 | 0.42 Rf | 45.0 AU | 0.51 Rf | 171.9 AU | 18.46 % | 0.55 Rf | 34.4 AU | 8724.5 AU | 21.46 % |
| 5 | 0.55 Rf | 134.8 AU | 0.62 Rf | 168.8 AU | 18.13 % | 0.65 Rf | 84.1 AU | 9048.9 AU | 22.26 % |
| 6 | 0.65 Rf | 84.2 AU | 0.72 Rf | 391.8 AU | 42.08 % | 0.77 Rf | 31.5 AU | 15569.8 AU | 38.29 % |
| 7 | 0.78 Rf | 31.7 AU | 0.81 Rf | 35.7 AU | 3.84 % | 0.88 Rf | 13.7 AU | 1738.5 AU | 4.28 % |

**Fig 2c. At 620nm (Post derivatisation)**

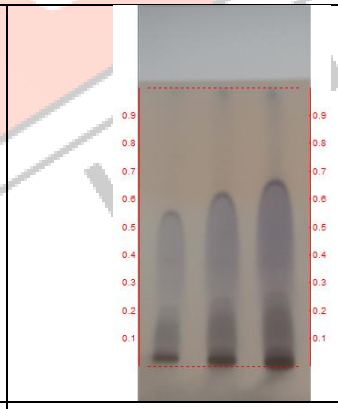
**Figure 3. HPTLC photo documentation of ethanolic extract of *Gokshura kwatha churna***



**Short UV**



**Long UV**



**Under white light (after derivatisation)**

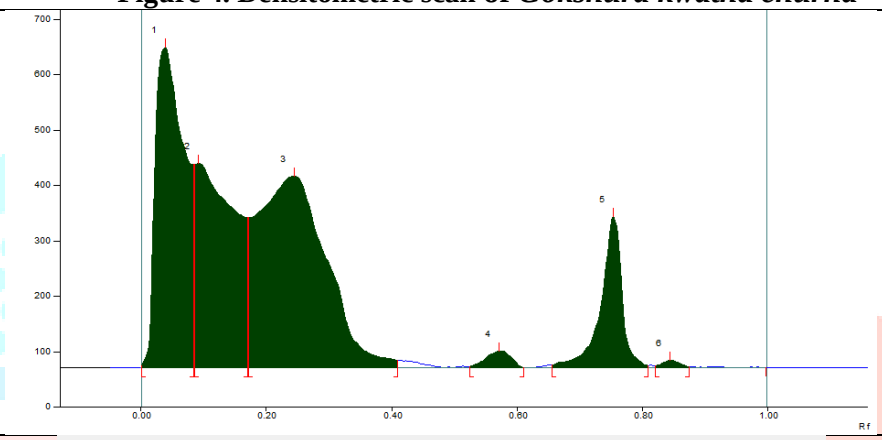
Track 1- ***Gokshura kwatha churna***– 3µl
Track 2- ***Gokshura kwatha churna***– 6µl
Track 3- ***Gokshura kwatha churna***– 9µl

**Solvent system – Toluene: Ethyl Acetate (8:1)**

**Table 4 : Rf value of *Gokshura kwatha churna***

| Short UV | Long UV | Under white light (after derivatisation) |
|---|---|---|
| 0.05 | - | - |
| - | 0.11 (F. blue) | - |
| 0.17 | - | 0.18 (Purple) |
| - | 0.20 (F. red) | - |
| - | - | 0.24 (Purple) |
| - | 0.26 (F. red) | - |
| - | 0.45 (F. red) | - |
| - | 0.53 (F. green) | - |
| - | 0.61 (F. blue) | - |
| - | 0.67 (F. red) | - |

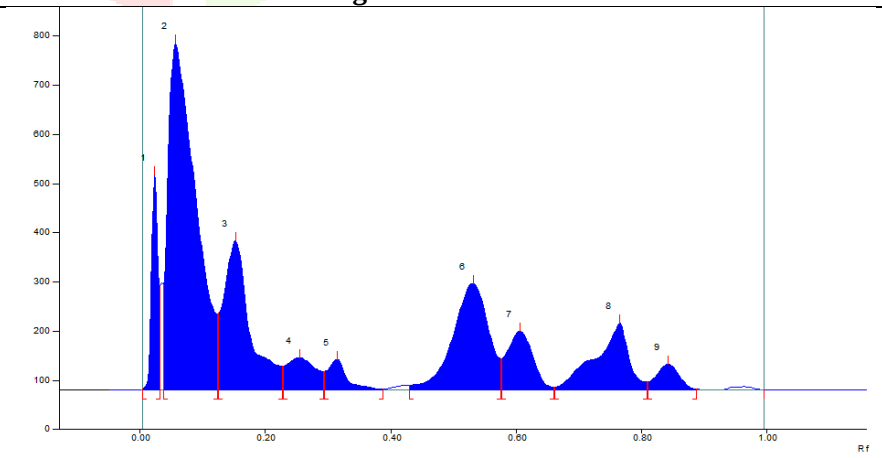
**Figure 4. Densitometric scan of *Gokshura kwatha churna***




Track 3, ID: Gokshura kwatha churna

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.00 Rf | 2.8 AU | 0.04 Rf | 579.0 AU | 35.97 % | 0.08 Rf | 66.4 AU | 19365.1 AU | 27.08 % |
| 2 | 0.09 Rf | 366.7 AU | 0.09 Rf | 368.2 AU | 22.87 % | 0.17 Rf | 70.9 AU | 17021.9 AU | 23.80 % |
| 3 | 0.17 Rf | 271.0 AU | 0.25 Rf | 345.3 AU | 21.45 % | 0.41 Rf | 12.8 AU | 27363.5 AU | 38.26 % |
| 4 | 0.53 Rf | 2.3 AU | 0.57 Rf | 30.5 AU | 1.90 % | 0.61 Rf | 0.5 AU | 892.1 AU | 1.25 % |
| 5 | 0.66 Rf | 4.7 AU | 0.75 Rf | 272.9 AU | 16.95 % | 0.81 Rf | 3.9 AU | 6597.8 AU | 9.23 % |
| 6 | 0.82 Rf | 3.4 AU | 0.85 Rf | 13.9 AU | 0.86 % | 0.88 Rf | 1.6 AU | 278.6 AU | 0.39 % |

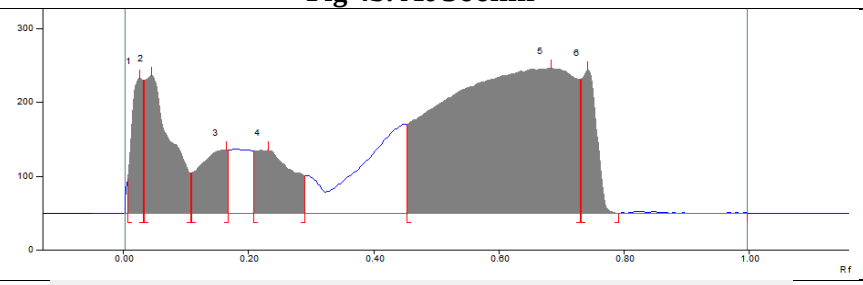
**Fig 4a. At 254nm**



Track 3, ID: Gokshura kwatha churna

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.00 Rf | 1.9 AU | 0.02 Rf | 436.5 AU | 20.84 % | 0.03 Rf | 10.1 AU | 3277.5 AU | 5.70 % |
| 2 | 0.04 Rf | 214.0 AU | 0.06 Rf | 704.4 AU | 33.62 % | 0.12 Rf | 54.2 AU | 22670.4 AU | 39.43 % |
| 3 | 0.13 Rf | 155.6 AU | 0.15 Rf | 303.2 AU | 14.47 % | 0.23 Rf | 48.0 AU | 9159.1 AU | 15.93 % |
| 4 | 0.23 Rf | 48.2 AU | 0.25 Rf | 65.7 AU | 3.13 % | 0.29 Rf | 36.9 AU | 2185.2 AU | 3.80 % |
| 5 | 0.30 Rf | 37.3 AU | 0.31 Rf | 62.2 AU | 2.97 % | 0.39 Rf | 1.4 AU | 1340.7 AU | 2.33 % |
| 6 | 0.43 Rf | 9.5 AU | 0.53 Rf | 216.2 AU | 10.32 % | 0.58 Rf | 64.0 AU | 8805.7 AU | 15.32 % |
| 7 | 0.58 Rf | 64.1 AU | 0.61 Rf | 119.0 AU | 5.68 % | 0.66 Rf | 5.6 AU | 3512.7 AU | 6.11 % |
| 8 | 0.66 Rf | 5.6 AU | 0.77 Rf | 135.4 AU | 6.46 % | 0.81 Rf | 16.0 AU | 5179.5 AU | 9.01 % |
| 9 | 0.81 Rf | 16.1 AU | 0.84 Rf | 52.6 AU | 2.51 % | 0.89 Rf | 1.1 AU | 1365.2 AU | 2.37 % |

**Fig 4b. At 366nm**




Track 3, ID: Gokshura kwatha churna

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.01 Rf | 46.4 AU | 0.03 Rf | 182.5 AU | 19.65 % | 0.03 Rf | 79.5 AU | 2431.4 AU | 5.11 % |
| 2 | 0.03 Rf | 179.6 AU | 0.05 Rf | 186.2 AU | 20.06 % | 0.11 Rf | 54.6 AU | 5577.0 AU | 11.72 % |
| 3 | 0.11 Rf | 54.9 AU | 0.16 Rf | 85.6 AU | 9.22 % | 0.17 Rf | 85.1 AU | 2751.0 AU | 5.78 % |
| 4 | 0.21 Rf | 84.0 AU | 0.23 Rf | 85.1 AU | 9.16 % | 0.29 Rf | 51.2 AU | 3685.1 AU | 7.75 % |
| 5 | 0.45 Rf | 120.7 AU | 0.68 Rf | 195.9 AU | 21.10 % | 0.73 Rf | 81.1 AU | 29786.0 AU | 62.60 % |
| 6 | 0.73 Rf | 181.3 AU | 0.74 Rf | 193.2 AU | 20.81 % | 0.79 Rf | 0.0 AU | 3347.4 AU | 7.04 % |

**Fig 4c. At 620nm (Post derivatisation)**

**Part D: Remarks**

The standardisation of *Shilodbidadi taila* and *Gokshura kwatha churna* was performed and results of which are given in Table 1. Phytochemical test performed qualitatively shows presence of alkaloids, glycosides, steroids, taanins, flavonoids, saponins, terpenoids, phenols and resins. HPTLC was performed for *Shilodbidadi taila* and *Gokshura kwatha* churna Rf values and densitometric scan are depicted in respective tables and figures.

## Discussion :

**Acid insoluble Ash**

To the crucible containing total ash, add 25ml of dilute HCl and boil. Collect the insoluble matter on ashless filter paper (Whatmann 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccator for 30 mins and weigh without delay. Calculate the content of acid insoluble ash with reference to the air dried drug.

**Alcohol soluble extractive**

Weigh accurately 4 g of the *Gokshura kwatha churna* in a glass stoppered flask. Add 100 ml of distilled Alcohol (approximately 95%). Shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours, cool in desiccator for 30 minutes and weigh. Calculate the percentage of Alcohol extractable matter of the sample. Repeat the experiment twice, and take the average value.

**Water soluble extractive**:

Weigh accurately 4 g of the *Gokshura kwatha churna* in a glass stoppered flask. Add 100 ml of distilled water, shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours. Cool in a desiccator and weigh. Repeat the experiment twice. Take the average value.

**HPTLC**

- 1g of *Gokshura kwatha churna* powder was extracted with 10 ml of alcohol . 3, 6 and 9µl of the above extract was applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (8.0: 1.0). The developed plates were visualized in short UV, long UV and then derivatised with vanillin sulphuric acid and scanned under 254nm, 366nm and 620nm. Rf, colour of the spots and densitometric scan were recorded.
- The standardisation of *Shilodbidadi taila* and *Gokshura kwatha churna* was performed and results of which are given in Table 1. Phytochemical test performed qualitatively shows presence of alkaloids, glycosides, steroids, taanins, flavonoids, saponins, terpenoids, phenols and resins. HPTLC was performed for Shilodbidadi taila and Gokshura kwatha churna .

The standardisation of *Shilodbidadi taila* and *Gokshura kwatha churna* was performed and results of which are given in Table 1. Phytochemical test performed qualitatively shows presence of alkaloids, glycosides, steroids, taanins, flavonoids, saponins, terpenoids, phenols and resins. HPTLC was performed for Shilodbidadi taila and Gokshura kwatha churna Rf values and densitometric scan are depicted in respective tables and figures.

## Conclusion

The presence of these elements in the final product is directly proportional to the biological activity expressed by the product. With this in mind the Qualitative analysis of the final product holds good. By understanding of different active principles present in different formulation helps in explaining their mode of action scientifically.

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