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ANALYTICAL STUDY AND STANDARDIZATION OF *KAKUBHADYA CHURNA*- A UNIQUE FORMULATION IN THE MANAGEMENT OF *RAJAYAKSHAMA*

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ABSTRACT

Ayurveda, the *Upaveda* of *Artharvana* deals with the holistic system of treatment. In *Bhaishajya Kalpana*, *Churna*, as an *Upkalpana* of *Kalka* is a concept which is highly accepted in *Ayurveda* offering a wide range of applicability. *Kakubhadya Churna* is one such herbal compound explained in *Brihat-Nighantu Ratnakara* under *Kshaya-Karma Vipaka Adhyaya* and is specifically indicated for *Kshaya* and *Kasa Roga*. **Aims and Objectives**: The primary aim is to standardize *Kakubhadya Churna* by performing various analytical tests. **Materials and Methods:** The study involves carrying out different analytical tests required as per standard testing protocol for *Churna Kalpana.* **Result:** The observations after each analytical test provide values helpful to standardize the *Churna*. **Discussion:** The study provides the analytical test results and observations which would tend to be useful in standardization of *Kakubhadya Churna* for further production. **Conclusion:** The study offers comprehensive analytical test results carried on *Kakubhadya Churna*.

KEYWORDS: *Kakubhadya Churna, Rajayakshama,* Analytical study, *Arjuna, Amalaki, Shunthi, Bala, Eranda Beeja, Ayurveda.*

INTRODUCTION

Rasashastra is defined as the branch of *Ayurveda* which deals with study of *Rasadi* Dravyas whereas *Bhaishajya Kalpana* deals with the preparation of medicine.

The *Sthavara* based pharmaceutics consider *Panchavidha Kashaya*[1] *Kalpana* under the basic preparation that forms the outline for secondary formulations in the name of *Upkalpana*. *Churna*, as an *Upkalpana* of *Kalka,* is the concept which is highly accepted in *Ayurveda* prepared with a single drug or combination of different drugs by homogenous mixing offering a wide range of applicability.

Kakubhadya Churna is mentioned in *Brihat-Nighantu Ratnakara* under *Kshaya-Karma Vipaka Adhyaya* and is specifically indicated for *Kshaya* and *Kasa Roga*.[2] The drugs have *Kshayaghna, Jwaraghna, Shwasaghna, Kasaghna, Sangrahi, Stambhana, Deepana, Daha-Prashamana, Vedana-Shamana* action and also have *Rasayana* effect.

Tuberculosis is a major health problem in the entire world. It is an infectious disease caused by *Mycobacterium tuberculosis*. Periodical development of drug-resistant traits in *Mycobacterium tuberculosis* has posed newer challenges in the treatment of the disease condition.[3] Owing to the similarities in symptoms and chronicity of *Rajayakshama* and Tuberculosis, this study is intended to standardize *Kakubhadya Churna* by performing various analytical tests which could be used for therapeutics.

AIMS AND OBECTIVES

The primary aim of this study is to standardize *Kakubhadya Churna* by performing various analytical tests.

MATERIALS AND METHODS

- Raw Drugs for the preparation were obtained from Sri Dharmasthala Manjunatheshwara Ayurveda Pharmacy Udupi.
- The Preparation of *Kakubhadya Churna* was carried out in *Rasashastra* and *Bhaishajya Kalpana* Pharmaceutical Study Lab, Sri Dharmasthala Manjunatheshwara College of Ayurveda, Hospital and Research Centre, Udupi.

Table 1: Ingredients of *Kakubhadya Churna.*

Drug name	Botanical name	Family name	Part used	Ratio[4]
Kakubha	*Terminalia arjuna*	Combrataceae	*Twak*	1 part
Bala	*Sida cordifolia*	Malvaceae	*Moola*	1 part
Amalaki	*Emblica officinalis*	Euphorbiaceae	*Phala*	1 part
Shunthi	*Zingiber officinale*	Zingiberaceae	*Kanda*	1 part
Eranda	*Ricinus communis*	Euphorbiaceae	*Beeja*	1 part

Figure 1: *Arjuna Twak.*

Figure 2: *Arjuna Twak Churna.*

Figure 3: Dried *Amlaki Phala.*

Figure 4: *Amalaki Churna.*

Figure 5: *Shunthi.*

Figure 6: *Shunthi Churna.*

Figure 7: ***Eranda Beeja.***

Figure 8: ***Eranda Beeja*** **and** ***Bala Moola Churna.***

Figure 9: ***Bala Moola.***

Figure 10: ***Kakubhadya Churna.***

ANALYTICAL TESTS

The analytical studies ensure quality, safety and efficacy of the formulation. In this study, organoleptic, physicochemical and chromatographic evaluation of *Kakubhadya Churna* were performed.

1. Organoleptic Characters[5]: *Kakubhadya Churna* was assessed based on its colour, odour, taste and consistency.
2. Determination of pH[5]:

- *Preparation of buffer solutions:*

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

- 1gm of *Kakubhadya Churna* was taken and made up to 100 ml with distilled water, stirred well and filtered. This was used for the experiment. Instrument was switched on and 30 minutes time was given for warming pH meter. The pH 4 solution was first introduced and the pH was adjusted by using the knob to 4.02 at room temperature 30°C. The pH 7 solution was introduced and the pH meter was adjusted to 7 by using the knob. The pH 9.2 solution was introduced and pH reading was noted without adjusting the

knob. The sample solution was introduced and reading was noted. The test was repeated four times and the average reading was taken as result.

3. Loss on drying at 105°C[5]

- 1 g of sample 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.

4. Total Ash[5]

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

5. Acid insoluble Ash[5]

- To the crucible containing total ash, 25ml of dilute HCl was added and allowed to boil. The insoluble matter was collected on ashless filter paper (Whatman 41) and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to attain a constant weight. The residue was allowed to cool in suitable desiccator for 30 mins and weighed without delay. The content of acid insoluble ash with reference to the air-dried drug was calculated

6. Water soluble ash[5]

- The ash was boiled for 5 min with 25 ml of water; insoluble matter was collected on an ashless filter paper, later washed with hot water and ignited for 15 min at a temperature not exceeding 450˚C. The weight of the insoluble matter was subtracted from the weight of the ash; the difference in weight represents the water soluble ash with reference to the air-dried sample.

7. Alcohol soluble extractive[6]

- Accurately weighed 4 g of the sample was taken in a glass stoppered flask. 100 ml of distilled Alcohol (approximately 95%) was added and shaken occasionally for 6 hours. It was allowed to stand for 18 hours. It was filtered rapidly taking care not to lose any solvent. 25ml of the filtrate was pipetted out in a pre-weighed 100 ml beaker and left to

evaporate till dryness on a water bath. Later, kept in an air oven at 105°C for 6 hours, cooled in desiccator for 30 minutes and weighed. The percentage of Alcohol extractable matter of the sample was calculated. The experiment was repeated twice and the average value was taken.

8. Water soluble extractive[6]

- Accurately weighed 4 g of the sample was taken in a glass stoppered flask. 100 ml of distilled water was added, shaken occasionally for 6 hours. Later, allowed to stand for 18 hours. It was filtered rapidly taking care not to lose any solvent. 25ml of the filtrate was pipetted out in a pre-weighed 100 ml beaker and evaporated till dryness on a water bath. It was kept in an air oven at 105°C for 6 hours and left to cool in a desiccator and weighed. The experiment was repeated twice and the average value was noted.

9. HPTLC[7]

- 1gm of *Kakubhadya Churna* was kept along with 10 ml methanol and kept for cold percolation for 24h and filtered. 3, 6 and 9µl of the above samples were applied on a pre-coated silica gel F_{254} on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate: Formic acid: Methanol (3:3:0.8:0.2). The developed plates were visualized in short UV, long UV and then derivatised with anisaldehyde sulphuric acid reagent and scanned under UV 254nm, 366nm, 540nm and 620nm. Retention factor, colour of the spots and densitometric scan were recorded.

10. Bulk density[8]

- The bulk density of a powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of the inter-particulate void volume. Hence, the bulk density depends on both the density of powder particles and the spatial arrangement of particles in the powder bed. The bulk density is expressed in grams per millilitre (g/mL). It may also be expressed in grams per cubic centimeter (g/cm3) when measurement is done in a graduated cylinder.

11. Tapped bulk density[8]

- The tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample. The tapped density is obtained by mechanically tapping a graduated measuring cylinder or vessel containing the powder sample. After

observing the initial powder volume or mass, the measuring cylinder or vessel is mechanically tapped, and volume or mass readings are taken until little further volume or mass change is observed. The mechanical tapping is achieved by raising the cylinder or vessel and allowing it to drop, under its own mass.

12. Haussners ratio[8]

- HR= V_0/V_f

13. Compressibility index[8]

- $CI = \frac{\rho tapped - \rho bulk}{\rho tapped} \times 100$

Table 2: Scale of Flowability.

Compressibility Index (%)	Flow character	Hausner's ratio
10	Excellent	1.00–1.11
11-15	Good	1.12-1.18
16-20	Fair	1.19-1.25
21-25	Passable	1.26-1.34
26-31	Poor	1.35-1.45
32-37	Very poor	1.46-1.59
>38	Very very poor	>1.60


Figure 11: *Kakubhadya Churna* and Aqueous extract of *KC*.

Figure 12: *KC* sample.

Figure 13: LOD using Muffle furnace.

Figure 14: Estimation of pH.

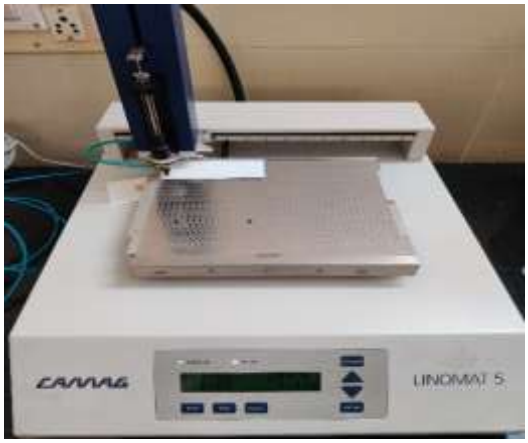


Figure 15: Sample application on a pre-coated silica gel F_{254} on aluminium plate.

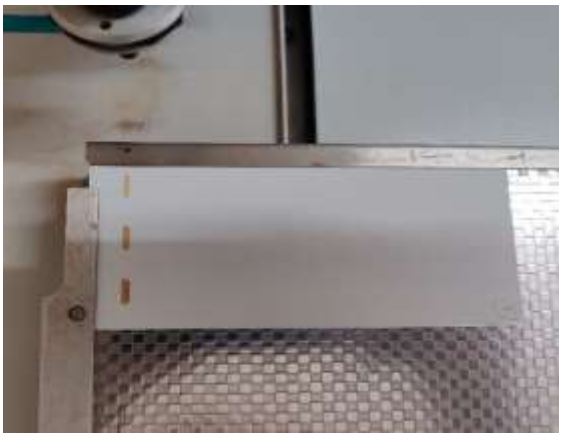


Figure 16: 3, 6 and 9µl of the *KC* sample applied on a pre-coated silica gel F_{254} on aluminium plate.

Figure 17,18: Developing Chamber with Toluene: Ethyl acetate: Formic acid: Methanol Solvent System.

Figure 19: Developed Plate for visualization.

Figure 20: Analytical Test.

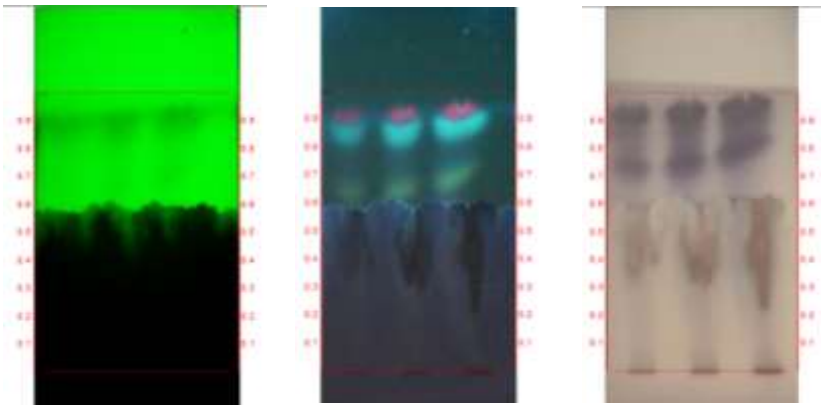
RESULT

Table 3: Results of Organoleptic Evaluation.

Parameter	Result
Colour	Greyish-Black
Odour	*Shunthi* Aroma
Taste	*Kashaya, Katu, Amla*
Consistency	Smooth Fine Powder

Table 4: Results of standardization parameters of *Kakubhadya Churna.*

Parameter	*Kakubhadya Churna*
pH	4.10
Loss on drying	12.01±0.00
Total Ash	12.93±1.31
Acid Insoluble Ash	2.96±0.01
Water soluble Ash	0.99±0.01
Alcohol soluble extractive value	39.95±0.07
Water soluble extractive value	29.98±0.03
Bulk density	0.416
Tapped bulk density	0.471
HR (Haussners ratio)	1.13 (Good flow)
CI (Compressibility index)	11.67 (Good)



Short UV **Long UV** **After derivatisation**

Figure 21: HPTLC photo documentation of Methanolic extract of *Kakubhadya Churna.*

Track 1- *Kakubhadya Churna* – 3µl

Track 2- *Kakubhadya Churna* – 6µl

Track 3- *Kakubhadya Churna* – 9µl

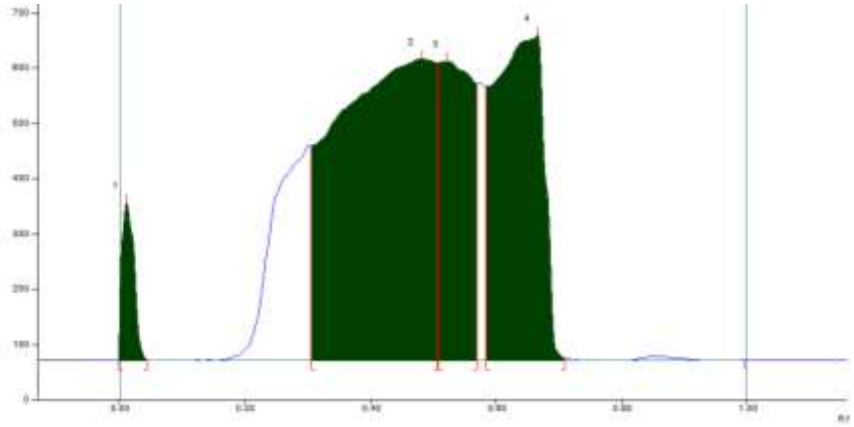
Solvent system – Toluene: Ethyl acetate: Formic acid: Methanol (3:3:0.8:0.2)

Rf 0.40 ± 0.08 - Gallic acid

Table 5: R_f values of samples.

Short UV	Long UV	After derivatisation
-	0.65 (F. green)	-
-	-	0.74 (Purple)
-	-	0.81 (Purple)
-	0.85 (F. blue)	0.86 (Purple)
-	0.91 (F. pink)	0.91 (Purple)

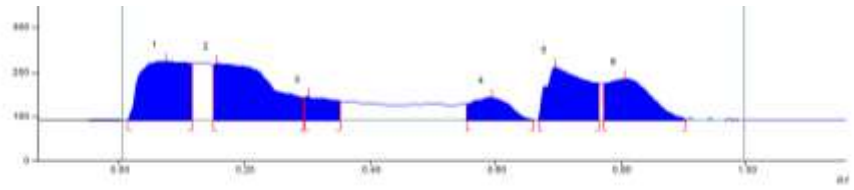
- F – fluorescent



Track 3, ID: Kakubhadya churna

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.01 Rf	286.1 AU	14.58 %	0.05 Rf	0.3 AU	3952.9 AU	3.31 %
2	0.31 Rf	388.4 AU	0.48 Rf	546.2 AU	27.84 %	0.51 Rf	38.1 AU	61411.8 AU	51.47 %
3	0.51 Rf	538.4 AU	0.52 Rf	541.3 AU	27.59 %	0.57 Rf	00.7 AU	20555.2 AU	17.23 %
4	0.59 Rf	495.2 AU	0.67 Rf	588.5 AU	29.99 %	0.71 Rf	3.9 AU	33403.7 AU	27.99 %

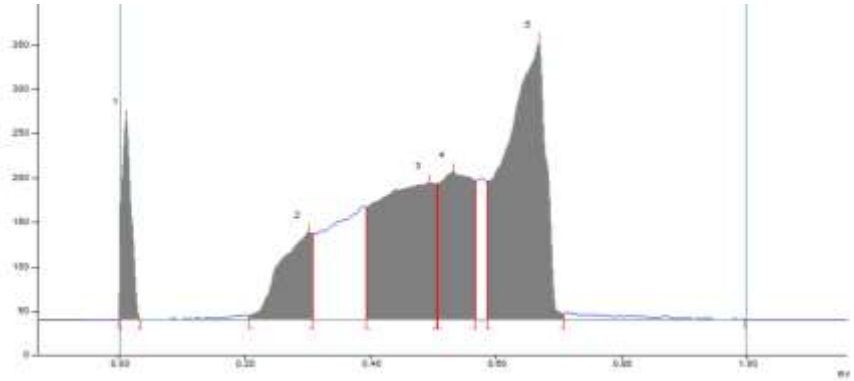
Figure 22: Densitometric scan of *Kakubhadya Churna.*



Track 3, ID: Kakubhadya churna

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.3 AU	0.07 Rf	132.6 AU	22.87 %	0.12 Rf	27.8 AU	7271.5 AU	23.93 %
2	0.15 Rf	126.6 AU	0.16 Rf	128.1 AU	22.09 %	0.30 Rf	51.8 AU	8763.2 AU	28.83 %
3	0.30 Rf	52.2 AU	0.30 Rf	54.3 AU	9.36 %	0.35 Rf	44.3 AU	1746.1 AU	5.75 %
4	0.55 Rf	37.3 AU	0.60 Rf	51.9 AU	8.96 %	0.66 Rf	2.1 AU	2280.3 AU	7.50 %
5	0.67 Rf	3.6 AU	0.70 Rf	119.5 AU	20.60 %	0.77 Rf	83.6 AU	5591.0 AU	18.40 %
6	0.77 Rf	83.5 AU	0.81 Rf	93.4 AU	16.12 %	0.90 Rf	2.0 AU	4739.0 AU	15.59 %

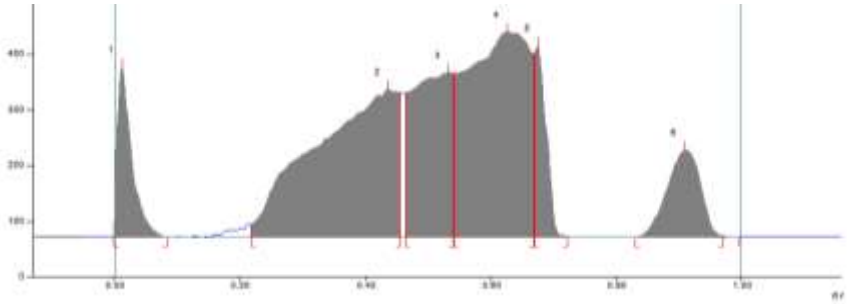
Fig. 22a: At 254nm. Rf 0.40 ± 0.08 (Gallic acid)



Track 3, ID: Kakubhadya churna

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.01 Rf	226.6 AU	23.63 %	0.04 Rf	0.1 AU	2333.3 AU	6.35 %
2	0.21 Rf	4.6 AU	0.30 Rf	98.4 AU	10.27 %	0.31 Rf	97.1 AU	3628.8 AU	9.87 %
3	0.39 Rf	126.8 AU	0.50 Rf	153.9 AU	16.06 %	0.51 Rf	52.7 AU	10241.1 AU	27.87 %
4	0.51 Rf	152.9 AU	0.53 Rf	166.6 AU	17.38 %	0.57 Rf	55.7 AU	6265.2 AU	17.05 %
5	0.59 Rf	155.9 AU	0.67 Rf	313.0 AU	32.66 %	0.71 Rf	6.4 AU	14280.0 AU	38.86 %

Fig 22b. At 366nm



Track 3, ID: Kakubhadya churna

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.01 Rf	305.8 AU	17.61 %	0.08 Rf	0.1 AU	5019.1 AU	6.22 %
2	0.22 Rf	22.4 AU	0.44 Rf	265.6 AU	15.29 %	0.46 Rf	59.2 AU	25220.2 AU	31.26 %
3	0.47 Rf	259.3 AU	0.53 Rf	296.2 AU	17.06 %	0.54 Rf	93.5 AU	13507.1 AU	16.74 %
4	0.54 Rf	293.5 AU	0.63 Rf	368.9 AU	21.25 %	0.67 Rf	29.2 AU	26327.5 AU	32.64 %
5	0.67 Rf	329.9 AU	0.68 Rf	343.2 AU	19.77 %	0.72 Rf	1.1 AU	4594.4 AU	5.70 %
6	0.83 Rf	0.1 AU	0.91 Rf	156.6 AU	9.02 %	0.97 Rf	2.1 AU	6002.7 AU	7.44 %

Fig 22c. At 540nm

DISCUSSION

Kakubhadya Churna is greyish-black in colour with a strong aroma of *Shunthi*. It has an astringent pungent taste due to presence of *Kashaya* and *Katu Rasa Dravya* and has slight *Amla Rasa* due to presence of *Amalaki*. *Churna* is a fine powder which is smooth to touch. *Kakubhadya Churna* has an acidic pH of 4.1 indicative of presence of an *Amla Dravya* which is *Amalaki* in the formulation. The result for LOD observed for *Kakubhadya Churna* was 12.01. Ash values refer to the inorganic residues left after complete incineration of organic matter. The Total Ash value observed for *Kakubhadya Churna* was 12.93 ± 1.31. The Acid insoluble ash value is indicative for the presence of silicaceous impurities and was noted to be 2.96 ± 0.01 for the sample. The Water-soluble ash value observed was 0.99 ±0.01 which is a qualitative standard used to determine the purity and authenticity of a sample. The Alcohol soluble extractive indicates the solubility of crude drug in water. The value obtained for *Kakubhadya Churna* was 39.95 ± 0.07. The Water-soluble extractive which tells about the proportion of biomass that is lost as a result of extraction with water was noted as 29.98 ± 0.03 for the formulation. The bulk density is the ratio of the mass of an untapped powder

sample and its volume including the contribution of the inter-particulate void volume which depends on both the density of powder particles and the spatial arrangement of particles in the powder bed. The bulk density for *Kakubhadya Churna* was 0.416. The tapped density which is an increased bulk density attained after mechanically tapping a container containing the powder sample was observed as 0.471 for the formulation. The Haussner's Ratio and Compressibility Index were observed as 1.13 and 11.67 respectively which is an indicative of good flow character of *Churna.*

HPTLC

Kakubhadya Churna was subjected for HPTLC to assess the phytochemical responsible for the probable mode of action of the formulation. The plate was developed in Toluene: Ethyl acetate: Formic acid: Methanol (3:3:0.8:0.2) solvent system specifically selected for the observation of Gallic Acid which has the Retention factor (Rf) of 0.40 ± 0.08. The developed plates were visualized in short UV, long UV and later derivatised with anisaldehyde sulphuric acid reagent and scanned which showed evident spots under UV. Spots were visualized at 0.65, 0.85 and 0.91 as fluorescent Green, fluorescent Blue and fluorescent Purple respectively under the long wavelength. After derivatisation, the spots were noted at 0.74, 0.81, 0.86 and 0.91 which were visualized in Purple colour but could not be distinctively identified. In densitometric scan, at 254 nm the area covered was 51.47% with Rf of 0.48 and at 620 nm wavelength, the area covered was 31.26% with Rf of 0.44 which is evident for the presence of Gallic acid.

CONCLUSION

The formulation was subjected for Analytical tests to standardize it according to the parameters applicable for *Churna Kalpana* and to generate a standard operative procedure for further production of *Churna*. The test result ensures the quality of the formulation making it fit for administration to the patients thereby providing a wide range of symptomatic relief from Tuberculosis and other respiratory ailments.

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