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Pharmacognostical evaluation of *Rajakoshataki* (*Luffa acutangula* (L.) Roxb.

Anju Balakrishnan[1], Mohammed Faisal[2], Suchitra Prabhu[3]

[1]*Post Graduate Scholar, Dept. of Dravya Guna, Shri Dharmasthala Manjunatheshwara College of Ayurveda, Udupi, Karnataka, India.*

[2]*Associate Professor, Shri Dharmasthala Manjunatheshwara College of Ayurveda, Udupi, Karnataka, India.*

[3]*Research Officer, SDM Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi, Karnataka, India.*

ABSTRACT

Introduction: *Rajakoshataki* (*Luffa acutangula*(L.) Roxb. is a succulent trailing climber that belongs to cucurbitaceae family. The present study attempts to evaluate macroscopy, microscopy, physio-chemical, phytochemical and HPTLC studies of different extracts of fruit of *Luffa acutangular* (L.)Roxb. **Materials and Methods:** Fresh *Rajakoshataki* fruit was collected from the local vegetable market, Udupi. Sample was preserved in fixative solution. The fixative used was FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml) for pharmacognostical study. Left sample were shade dried for preliminary phytochemical test, physiochemical and HPTLC. **Results and discussion:** Microscopic image showed the presence of normal fruit structure like Epicarp, Mesocarp and Endocarp. The study proved the presence of Alkaloids, Tannins, Saponins and carboxylic acid and HPTLC densitometric graph showed the peaks. **Conclusion:** These parameters help in the identification and standardization of fruit of *Rajakoshataki*.

Key words: *Rajakoshataki, Physio-Chemical, Phytochemical, HPTLC, Densitometric*

INTRODUCTION

India is rich in its natural diversity of plant kingdom. There is no herb devoid of medicinal value. Lack of scientific evidence leads to decline of Ayurvedic treatment modality. But now a days there are high demand for herbal drugs because of the less side effects and high therapeutic efficacy with its easy availability.

Address for correspondence:
Dr. Anju Balakrishnan
Post Graduate Scholar, Dept. of Dravya Guna, Shri Dharmasthala Manjunatheshwara College of Ayurveda, Udupi, Karnataka, India.
E-mail: anjubalakrishnan333@gmail.com

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Rajakoshataki (*Luffa acutangular* (L.) Roxb. Belongs to Cucurbitaceae family and is commonly known as Ridge gourd in English. It is a succulent, trailing, decumbent annual or perennial herb. It climbs by means of laterally spirally coiled, simple or branched tendrils.[1] It is cultivated throughout India and widely used as vegetable crop. In folklore medicine the fruit of *Rajakoshataki* is widely used in many ailments like jaundice, fever, hyperuricemia[2] etc. Fruits and tender leaves are widely used for therapeutic purpose as well as diet. But adulteration and substitution will cause a barrier for this. Hence this study is an attempt to develop standardization parameters including macroscopy, microscopy, photochemical physiochemical and HPTLC of *Rajakoshataki* fruit.

MATERIALS AND METHODS

Drug source: The fruit of *Rajakoshataki* is collected from local vegetable market of Udupi. The drug is authenticated and standardization is done in the Pharmacognostic lab of SDM center for Research and Allied Sciences, Udupi.

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Macroscopy

The external features of the test samples were documented using Canon IXUS digital camera. The macroscopic features were compared to local flora for authentication.

Microscopy

Sample was preserved in fixative solution. The fixative used was FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). The materials were left in FAA for more than 48 hours. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with saffranine. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.

Physico-chemical standards[3]

The sample is tested for Loss on drying, Total ash, Water soluble ash, Acid insoluble ash, Alcohol soluble extractive and water soluble extractive as per standard protocol.

Phytochemical study[4]

Alcohol and aqueous extract of the drug was used to check the presence of secondary metabolites like alkaloids, carbohydrates, steroids, saponins, tannins, flavanoids, phenol, coumarins, triterpinoids, carboxylic acid, resin and quinone.

HPTLC[5,6]

One gram of powdered sample of fruit of *Luffa acutangula* were suspended in 10 ml methanol (99.9%) 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 Chloroform: Hexane: Methanol: Formic acid (6.4: 3.9: 2.0: 0.5). The developed plates were visualized in short UV, long UV and then derivatized with Vanillin sulphuric acid reagent (VSA) and scanned under UV 254nm, 366nm and 620nm. Rf, colour of the spots and densitometric scan were recorded.

Observations and Results

Organoleptic features

Colour **:** Dark green

Size : 10 to 30 cm

Taste : Sweet - bitter

Odour : Characteristic odour

Macroscopy

Pepo, obovate, having 10 acute lines on it, which is from base to apex of the fruit. Fruit is narrow towards both ends and broader in the middle. Matured fruits are used as natural sponge.

Seed - Ovate to oblong, dorsiventrally flattened, colour chocolate brown to black.

Figure 1: Macroscopy of fruit

Ridge guard (*Luffa acutangula* fruit)

Microscopy

Transverse section of fruit shows all the general microscopic characteristics of fruit. i.e., presence of epicarp, mesocarp and endocarp, which are well differentiated.

Epicarp: It is the outer most layers of fruit made up of thin rectangular cells with a thick cuticle and stomata at regular intervals. In mature fruits there are layers of subepidermal collenchyma.

Mesocarp: It is made up of many layers of thin compactly arranged parenchymatous cells, shows lignified fibers and scleranchymatous cells and layer of lignified cells below epicarp are present in parenchyma. Vascular tissue is also observed in parenchymatous region which is partially covered with

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lignified fiber. Starch grains are also present. The lignified fibers are scattered in mesocarp.

Endocarp: It is made of simple large polygonal parenchymatous cell which envelops the seeds. These are partially lignified and sclerenchymatous cells are scattered.

Figure 2: Microscopy of fruit of *Luffa acutangular.*

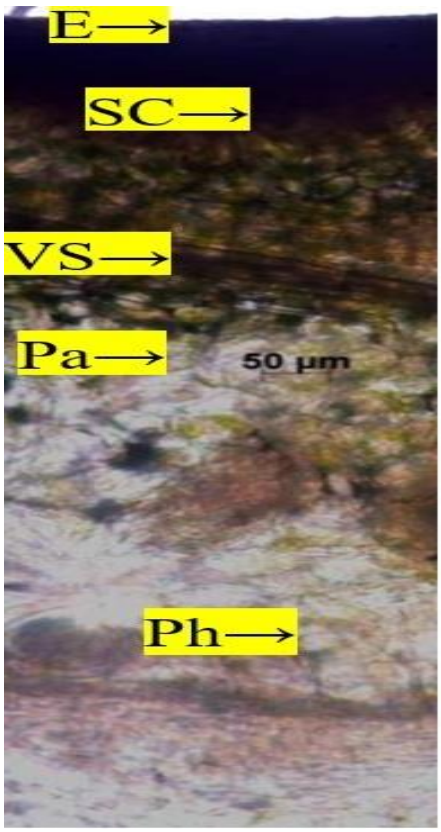

Fig 2a. Epicarp and Mesocarp

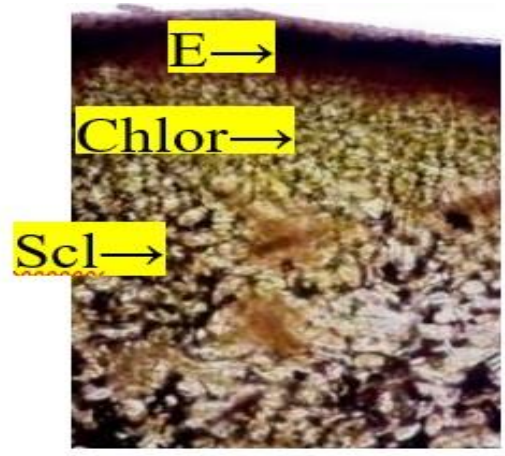

Fig 2b. Mesocarp enlarged

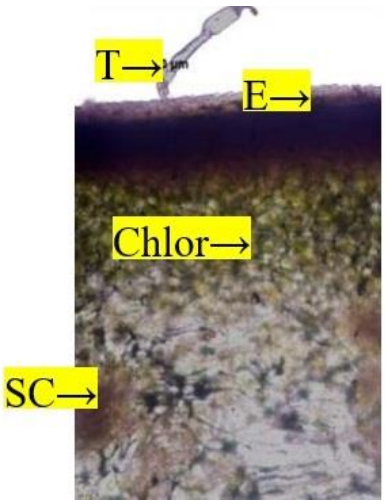

Fig 2c. Stone cells and Sclereids zone

E-epicarp; Chlor–chlorenchyma; Pa-parenchyma; Ph-phloem; SC – stone cells; Scl–sclereids; T–trichomes; VS-vascular strands.

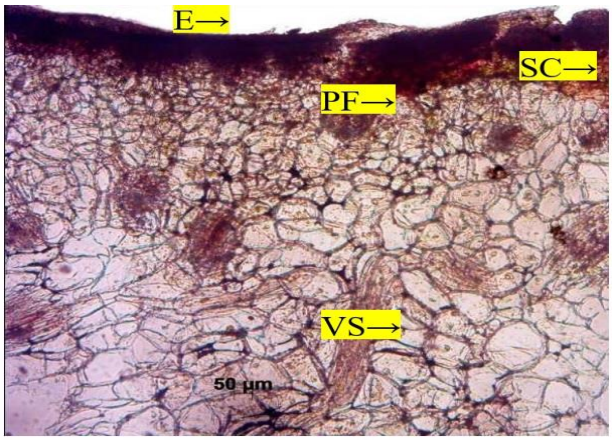

Fig 2d. Epicarp and mesocarp

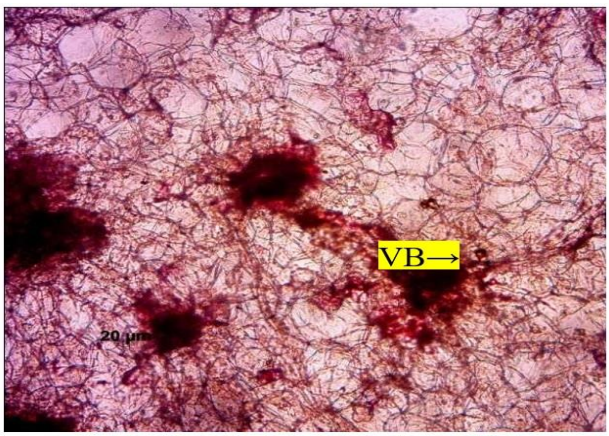

Fig 2e. Scattered vascular bundle

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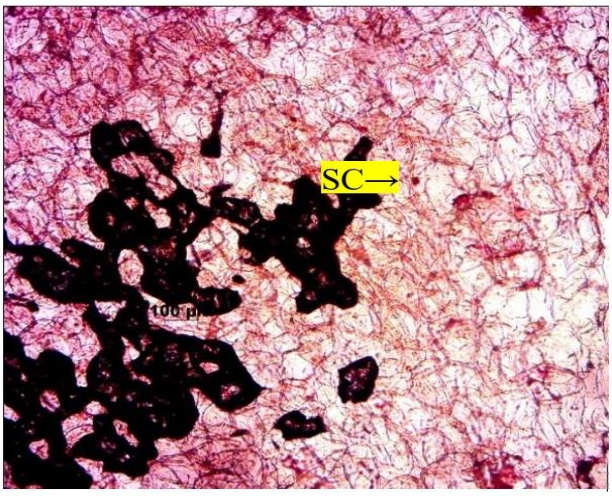

Fig 2f. Stone cells

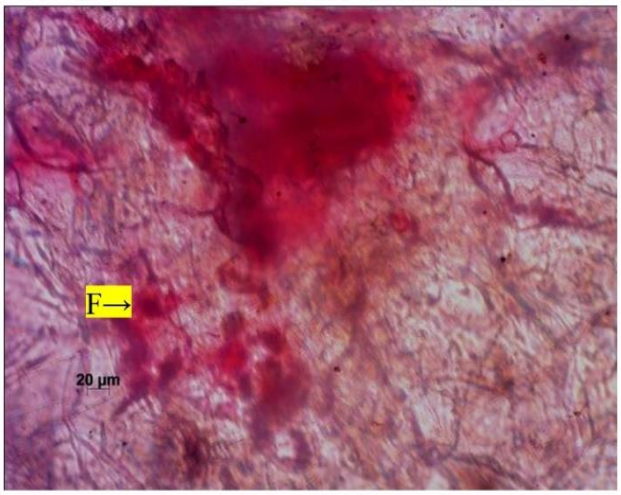

Fig 2g. Lignified cells

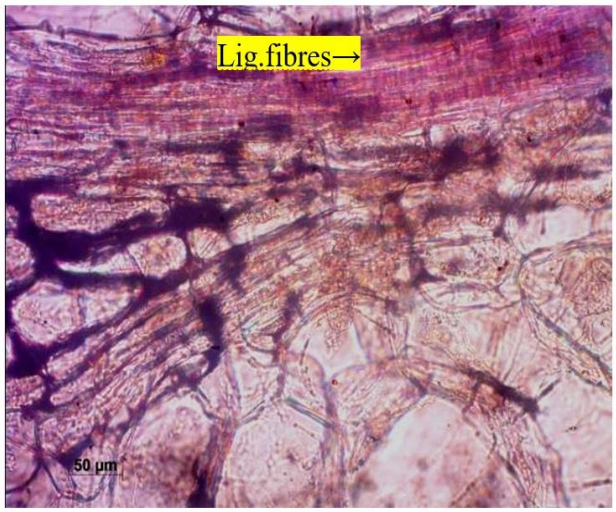

Fig 2h. Vascular strand /Lignified fibres

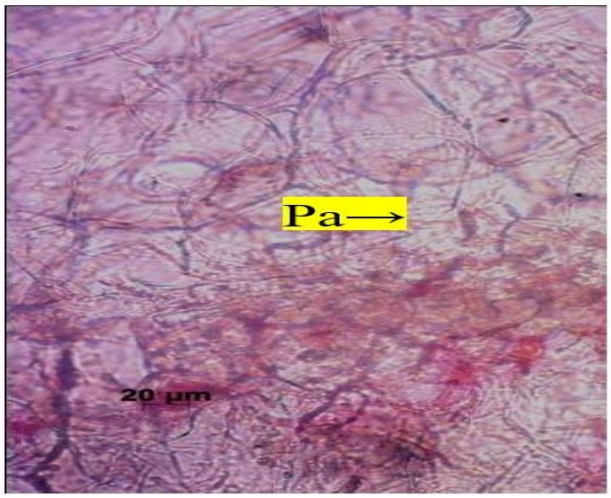

Fig 2i. Ground tissue

E-epicarp; F- fibres; Lig.fibres-lignified fibres; Pa-parenchyma; SC – stone cells; VS – vascular strands. VB–vascular bundles

Physico-chemical standards

Table 1: Results of Physico-chemical parameters of fruit of *Luffa acutangular.*

Parameter	Results n = 3 %w/w Avg ± SEM
Loss on drying	30.06 ± 0.00
Total Ash	5.67 ± 0.02
Acid Insoluble Ash	0.56 ± 0.01
Water soluble Ash	5.49 ± 0.01
Alcohol soluble extractive value	7.46 ± 0.01
Water soluble extractive value	40.94 ± 0.05

Table 2: Results of preliminary phytochemical screening of fruit juice of *Luffa acutangula*

Test	Inference
Alkaloid	+
Steroid	-
Carbohydrate	-
Tannin	+

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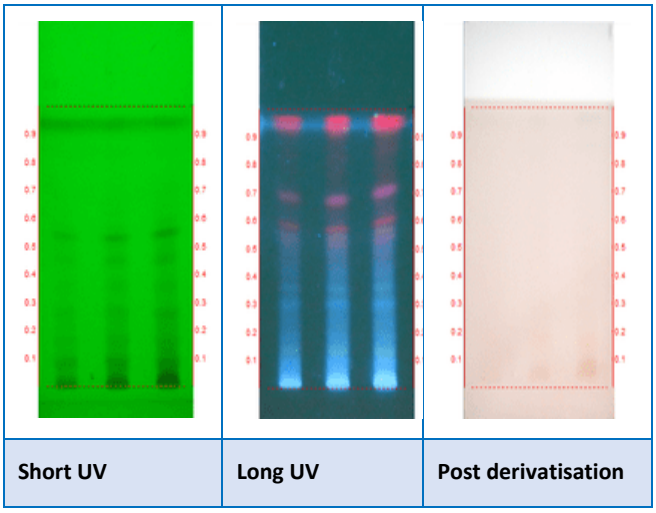
Flavanoids	-
Saponins	+
Terpenoid	-
Coumarins	-
Phenols	-
Carboxylic acid	+
Amino acids	-
Resin	-
Quinone	-

(+) – Present; (-) – Negative

Figure 3: HPTLC photo documentation of methanolic fraction of *Luffa acutangula*



Short UV	Long UV	Post derivatisation

Solvent system - Chloroform: Hexane: Methanol: Formic acid (6.4:3.9: 2.0: 0.5)

Track 1 – ***Luffa acutangula*** – 3µl

Track 2 – ***Luffa acutangula*** – 6µl

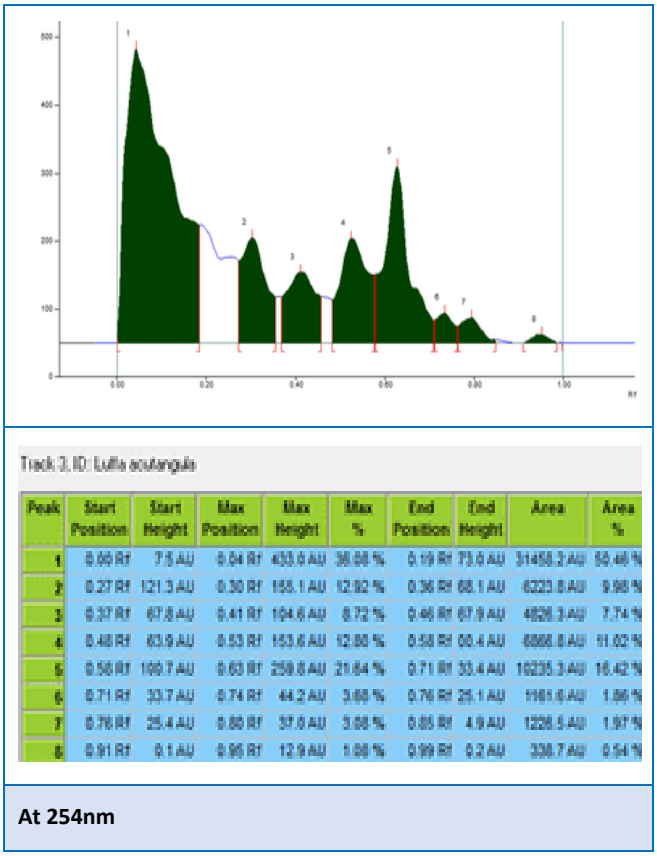
Track 3 – ***Luffa acutangula*** – 9µl

Table 3: R_f values of *Luffa acutangula*

Short UV	Long UV	Post derivatisation
-	-	0.06 (Brown)
0.09 (Green)	-	-
-	0.13 (F. green)	-
0.16 (Green)	-	0.17 (Brown)
0.28 (Green)	-	-
-	0.30 (F. blue)	-
0.36 (Green)	0.36 (F. blue)	-
0.45 (Green)	-	-
-	0.49 (F. blue)	-
0.52 (Green)	0.52 (F. pink)	-
-	0.54 (F. pink)	-
-	0.57 (F. red)	-
-	0.68 (F. pink)	-
-	0.94 (F. red)	-
-	0.96 (F. red)	-

*F- fluorescent

Figure 4: Densitometric scan for the methanolic extract of fruit of *Luffa acutangula*



At 254nm

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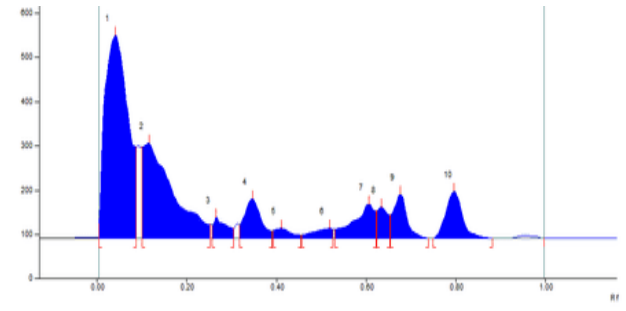
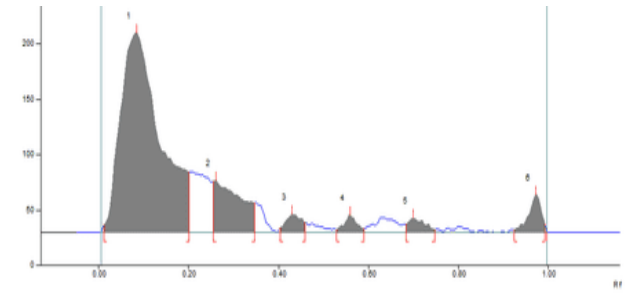


Track 3, ID: Luffa acutangula

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	33.9 AU	0.04 Rf	456.4 AU	38.63 %	0.09 Rf	67.4 AU	17006.4 AU	41.51 %
2	0.10 Rf	206.9 AU	0.12 Rf	214.4 AU	17.79 %	0.25 Rf	31.3 AU	10576.9 AU	25.82 %
3	0.26 Rf	31.5 AU	0.27 Rf	46.8 AU	3.89 %	0.30 Rf	22.4 AU	997.1 AU	2.43 %
4	0.32 Rf	30.3 AU	0.35 Rf	88.5 AU	7.34 %	0.39 Rf	17.1 AU	2364.1 AU	5.75 %
5	0.39 Rf	17.3 AU	0.41 Rf	22.0 AU	1.83 %	0.45 Rf	7.1 AU	607.7 AU	1.48 %
6	0.46 Rf	7.3 AU	0.52 Rf	22.1 AU	1.84 %	0.53 Rf	21.5 AU	712.8 AU	1.74 %
7	0.53 Rf	21.3 AU	0.61 Rf	77.5 AU	6.43 %	0.62 Rf	61.4 AU	2575.2 AU	6.29 %
8	0.62 Rf	61.9 AU	0.64 Rf	69.4 AU	5.76 %	0.65 Rf	52.6 AU	1190.2 AU	2.90 %
9	0.65 Rf	52.9 AU	0.68 Rf	100.0 AU	8.29 %	0.74 Rf	0.2 AU	2142.0 AU	5.23 %
10	0.75 Rf	0.6 AU	0.80 Rf	106.1 AU	8.80 %	0.88 Rf	0.2 AU	2808.5 AU	6.85 %

At 366nm



Track 3, ID: Luffa acutangula

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	6.5 AU	0.08 Rf	180.4 AU	58.90 %	0.29 Rf	54.8 AU	11484.0 AU	74.98 %
2	0.25 Rf	46.3 AU	0.26 Rf	47.2 AU	15.42 %	0.35 Rf	26.7 AU	2056.3 AU	13.43 %
3	0.40 Rf	4.2 AU	0.43 Rf	16.3 AU	5.33 %	0.46 Rf	6.8 AU	413.0 AU	2.70 %
4	0.53 Rf	2.7 AU	0.56 Rf	15.6 AU	5.11 %	0.59 Rf	3.6 AU	323.5 AU	2.11 %
5	0.68 Rf	7.1 AU	0.70 Rf	12.9 AU	4.21 %	0.75 Rf	2.4 AU	352.2 AU	2.30 %
6	0.90 Rf	2.6 AU	0.97 Rf	33.8 AU	11.03 %	0.99 Rf	7.8 AU	687.7 AU	4.49 %

At 620nm

DISCUSSION

Standardization helps in the authentication of the herbal drug in association with public health issues. It helps to identify the instances where the drug is adulterated/substituted. It ensures the identity and purity of licensed herbal drugs.

Macro-microscopy of the drug brings out the comprehensive identity profile of the fruit of *Rajakoshataki*. Loss on drying (30.06 ± 0.00) is used to determine the moisture content of the drug. Total ash value (5.67 ± 0.02) is obtained after complete combustion of drug sample. It is the measure of inorganic impurities in the sample. It gives estimation about the purity and quality of the drug. Acid Insoluble Ash (0.56 ± 0.01) indicates the presence of fine soil and sand particles present in the sample. Water soluble Ash (5.49 ± 0.01) is the part of total ash dissolved by water under specified conditions. It gives the estimation of inorganic contents in the sample. Alcohol soluble extractive value (7.46 ± 0.01) is primarily used for the determination of exhausted or adulterated drugs. It plays an important role in determining the identity and purity of the drug. Water soluble extractive value (40.94 ± 0.05) also plays an important role in the evaluation of crude drug. It indicates the addition of exhausted or adulteration of drugs. The Phytochemical tests indicate the presence of high amount of alkaloids, tannins, saponins and carboxylic acid.

Test drug sample when developed on aluminum plates having media Chloroform: Hexane: Methanol: Formic acid (6.4: 3.9: 2.0: 0.5), 6 peaks were observed on short UV, 10 peaks in long UV and 2 peaks post derivatization with Vanillin sulphuric acid reagent.

CONCLUSION

Rajakoshataki - *Luffa acutangular* (L.)Roxb. is a succulent, trailing, decumbent annual or perennial climber which belongs to cucurbitaceae family. It is one of the widely used vegetable crop which is cultivated throughout India. This study is an attempt to develop multidimensional identification characteristics of *Rajakoshataki* fruit including macroscopic, microscopic, physiochemical, preliminary phytochemical profile and HPTLC.

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