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# Analytical profile of *Kushtha (Saussurea lappa)* extensively used medicinal plant in Ayurveda system of medicine

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## ABSTRACT

*Ayurveda* is one of the most ancient medical sciences of the world. It is considered as the *Upaveda* of *Atharvaveda*. *Kushtha (Saussurea lappa - C.B.Clarke)* has been mentioned in various texts of *Ayurveda*. *Acharya Charaka* has incorporated *Kushtha* in more than 100 formulations. It is called similar activity as divine plant *Soma*. It grows nearby *Soma* in Himalayas. *Kushtha (Saussurea lappa - C.B.Clarke)* is used for the treatment of disease and novel drug entities continue to be developed through research into their constituents. So much adulteration and sub standard quality drug samples are found in the name of *Kushtha (Saussurea lappa - C.B.Clarke)* in the market. Analytical and physicochemical profiles for standardization of medicinal plants is need of hour. *Costunolide* can be marker compound for the identification of *Kushtha (Saussurea lappa - C.B.Clarke.)*

**Key words:** *Ayurveda, Saussurea lappa, Medicinal Plant, Analysis.*

## INTRODUCTION

Standardization of the medicines of ISM is the need of the day and is gaining more and more attention from drug control authorities, researchers and manufactures of this system. Moreover to globalize these medicines strict quality control parameters are to be followed for their analysis. Phyto-chemistry is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with chemical structures of those substances, their biosynthesis, turn over and

metabolism, their natural distribution and their biological function.<sup>[1]</sup>

*Ayurveda* is one of the most ancient medical sciences of the world. It is considered as the *Upaveda* of *Atharvaveda* and has thus its origin from Vedas, the oldest recorded wisdom on the earth. It is survived through two sets of original authentic texts each consisting of three books like *Bruhatrayi* and *Laghutrayi*.

With passing of hundreds of year, some drugs were disappeared from routine practice. *Kushtha* is one of them, which has been mentioned since from *Vedic Kala (Atharvaveda)* to latest modern literature available about plants. First authentic reference about this plant is found in *Atharvaveda*.<sup>[2]</sup>

It is called similar activity as divine plant *Soma*. It grows nearby *Soma* in Himalayas. Almost all actions of this plant are similar to divine plant *Soma*. It was used in Vedic rituals as well as in health preventive and primitive practice in Vedic period. In Vedic literature *Kushtha* is described with three synonyms viz., *Nadamara, Nadyarisa* and *Nadyaya*. *Nadya* is

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mentioned in *Atharvaveda*, *Sounakiya Paippalada Shakha*. In Vedas *Kushtha* is claimed to cure several diseases and possess the properties like; *Rasayana*, *Vrushya* and *Krimighna*. It is specifically mentioned as *Takmanashaka* (anti-pyretic).<sup>[3]</sup>

*Kushtha* has been mentioned in various texts not in single heading but in scattered manner.<sup>[4]</sup> *Acharya Charaka* has incorporated *Kushtha* in more than 100 formulations.<sup>[5]</sup>

*Kushtha (Saussurea lappa. – C.B.Clarke)* is used for the treatment of disease and novel drug entities continue to be developed through research in to their constituents. So much adulteration and sub standard quality drug samples are found in the name of *Kushtha (Saussurea lappa. – C.B.Clarke)* in the market.<sup>[6]</sup> This study is undertaken with following aims and objectives.

### AIMS AND OBJECTIVES

1. To analyze the samples by using different physico-chemical parameters and qualitative methods.
2. To develop the TLC profile and HPTLC profile.

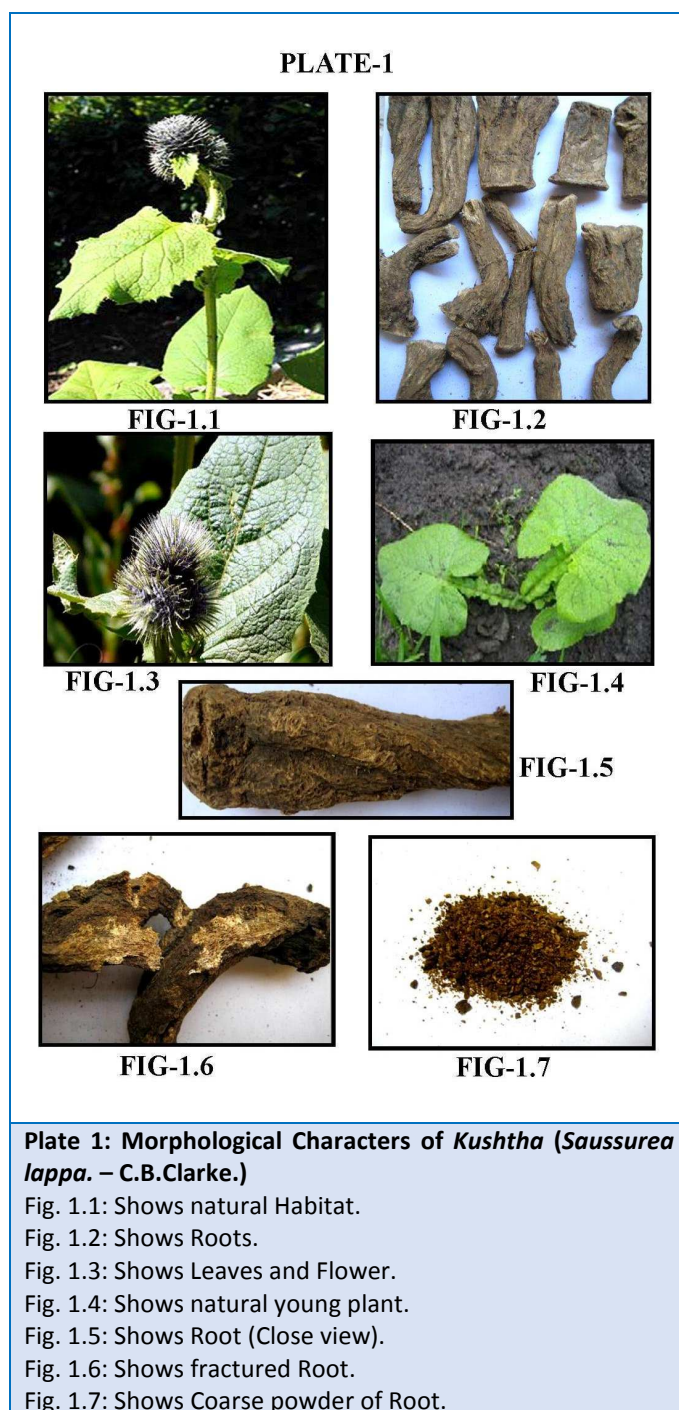
### MATERIALS AND METHODS

The test drug *Kushtha (Saussurea lappa. – C.B.Clarke)* was collected from Chakarata Deharadun (India) and after being authenticated botanically by expert of I.P.G.T. & R.A. Jamnagar, it was used for further analysis.

Comparative parameters have been used as per Ayurvedic Pharmacopoeia of India (API) Part-1-Vol.-1-Guidelines.

### Extraction

Two different extraction procedures were adopted for the analysis. Water-soluble extractive principle: this extract was used for qualitative analysis. Another extraction was done by using methanol soluble principle. The selected extract was compared with a drug preparation treated with heat to dry and selection of methanol soluble was taken for chromatographic study.



### Physico-Chemical Parameters

- Loss on drying
- Ash value
- Water soluble extractive
- Methanol soluble extractive

### Qualitative Analysis

- Alkaloids
- Flavonoids
- Glycoside

- Phenol
- Amino acid
- Protein
- Tannin
- Carbohydrate

### Thin Layer Chromatography (TLC)

Thin layer chromatography on Silica Gel in normal phase mode.

### HPTLC Profile

On Aluminium backed Silica Gel GF 254 in normal phase.

### Densitometry

Using Camag TLC Scanner 3

### Peak Assignment and UV-Vis Spectrum

In situ scanning mode using Camag Scanner 3.

### Methods

Physico-Chemical Parameters,<sup>[7]</sup> Qualitative Tests For Various Functional Groups,<sup>[8]</sup> Thin Layer Chromatography (TLC),<sup>[9]</sup> HPTLC Profile were done as per standard techniques available in Ayurvedic Pharmacopoeia of India, (2001) (1st ed., Govt. of India, Ministry of Health and Family Welfare, Dept. of Indian Systems of Medicine & Homeopathy, New Delhi) Part - I, and Quality Standards of Indian Medicinal Plants.(2006) (Indian Council of Medicinal Research, Delhi - Vol- 4 pg.no.204.)

## OBSERVATION AND RESULTS

### Physio-Chemical Parameters

The analytical data of common Physico-chemical parameters of the samples is tabulated in the table below

**Table 1: Physico-chemical parameters of the samples.**

| SN | Test           | Sample 1-<br><i>Kushtha</i> | <i>Kushtha</i> (Root)<br><i>Churna</i> (API) |
|----|----------------|-----------------------------|--|
| 1. | Loss on drying | 0.49% w/w                   | Not more than 2 %                            |
| 2. | Ash value      | 3.25% w/w                   | Not more than 4 %                            |

|    |                          |            |                    |
|----|--------------------------|------------|--------------------|
| 3. | Water soluble extract    | 22.38%w/w  | Not less than 20 % |
| 4. | Methanol soluble extract | 13.45% w/w | Not less than 12 % |

The table includes the parameters that affect the storage condition of drug and raw material processing and primary information about the drug tested.

Loss on drying reflects the nature of powder to hold moisture. It is assumed that high moisture holding capacity directly reflects the stability as carbohydrate and similar components provide natural growing atmosphere to microorganism. *Kushtha* have no quotable change as 0.49% w/w was observed. Like loss on drying, drug have Ash content i.e. 3.25% w/w for *Kushtha*, In general, the data gives information about total inorganic load. For further comparison ash analysis is suggested for specific positive and negative elements.

In extractive principle, *Kushtha* shows high water soluble components i.e. 22.38%w/w . While methanol solubility was found 13.45% w/w in *Kushtha*.

### Qualitative Tests

The samples were qualitatively tested for the presence of different phyto- constituents and the result has been presented in table below,

**Table 2: Qualitative tests of powder samples.**

| SN | Tests      | Name of reagent       | Results |
|----|------------|-----------------------|---------|
| 1  | Alkaloids  | Wagner's reagent      | +       |
|    |            | Dragendorff's reagent | +       |
| 2  | Flavonoids | Lead acetate          | -       |
|    |            | Shinoda test          | -       |
| 3  | Glycoside  | Legal's test          | +       |
|    |            | Keller Killiani       | +       |

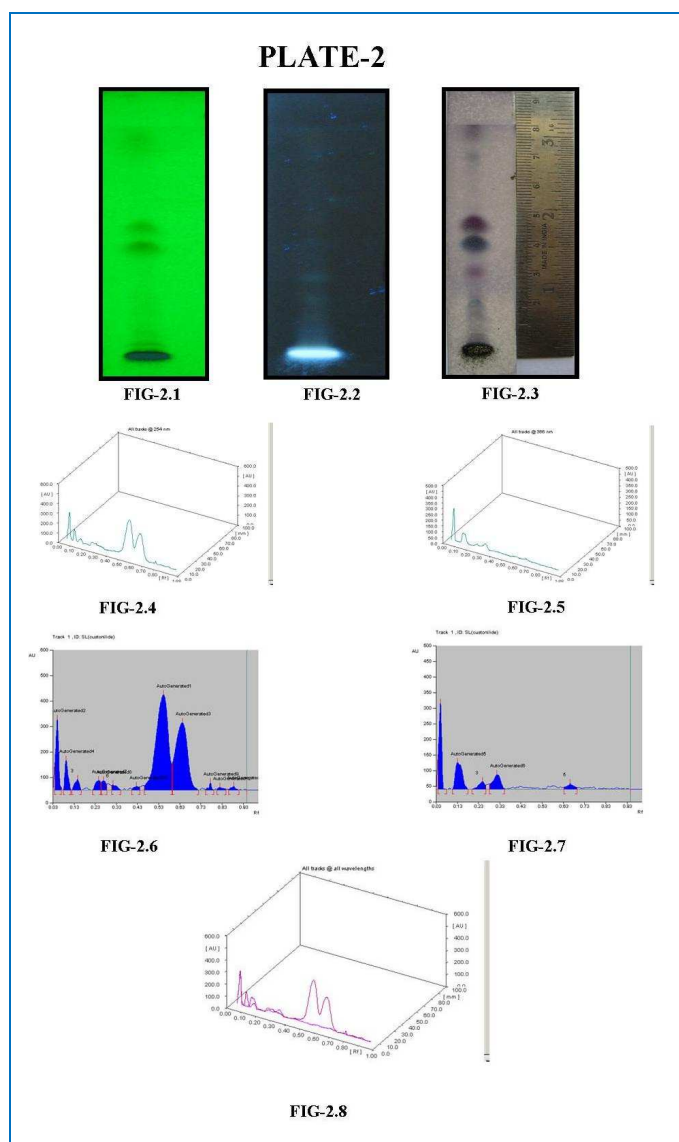


|                                 |              |   |   |
|---------------------------------|--------------|---|---|
| 4                               | Phenol       | NH <sub>4</sub> OH + potassium ferricyanide | - |
|                                 |              | Dilute potassium permanganate               | - |
| 5                               | Amino Acid   | Ninhydrin test                              | - |
| 6                               | Protein      | Biuret test                                 | - |
|                                 |              | Milion's test                               | - |
| 7                               | Tannin       | Lead acetate                                | + |
|                                 |              | Ferric chloride                             | + |
| 8                               | Carbohydrate | Molisch test                                | - |
|                                 |              | Fehling's test                              | - |
| +ve - Positive, - ve - Negative |              |   |   |

The qualitative test for Alkaloids with Wagner's reagent and Dragendroff's reagent was positive. Shinoda test and Lead acetate test for Flavonoids was found to be negative. Tannins are present in *Kushtha (S.lappa)* when tested with lead acetate and ferric chloride. Keller Killiani test and Legal test were positive. Which indicate presence of glycosides. NH<sub>4</sub>OH + potassium ferricyanide test and potassium permanganate test for phenols were found to be negative. Ninhydrin test for Amino acids whereas Biuret test and Milion's test for proteins was found to be negative for *Kushtha (S.lappa)* For the detection of Carbohydrate, Fehling's test and Tollen's test gave negative for *Kushtha (S.lappa)* Thus it can be seen that Alkaloids, Glucosides are present in *Kushtha (S.lappa)* Tannins are present in *Kushtha (S.lappa)*.

### Thin Layer Chromatography

Thin layer chromatographic work confirms separation of components, hence different solvent systems were tested for good separation, then the same protocols were followed in HPTLC fingerprinting.



**Plate 2: TLC & HPTLC Kushtha (*Saussurea lappa* – C.B.Clarke) extract under short and long UV radiation.**

#### Chromatographic condition

**Stationary phase:** Silica gel G F 254

**Mobile phase:** Toluene: Ethyl acetate (97: 3)

**Detection:** (i) Short UV (254nm) (ii) Long UV (366nm)

Fig. 2.1:TLC separation visualization under short UV. (Rf- 0.37)

Fig.2.2: TLC separation visualization under long UV. (Rf- 0.37)

Fig. 2.3: Visualization after Spray with Anisaldehyde-Sulphuric acid reagent. (Rf- 0.37).

Fig. 2.4: Chromatographic separation of Extract under short UV

Fig. 2.5: Chromatographic separation of Extract under long

Fig. 2.6: Densitogram of Extract under short UV

Fig. 2.7: Densitogram of Extract under long UV

Fig. 2.8: Chromatographic separation of Extract at all wavelengths.

HPTLC Profiling<sup>[10]</sup>**Table 3: Showing HPTLC profile with mobile phase Toluene: Ethyl acetate (97: 3)**

|                | Detection under short UV radiation (254 nm) |                  | Detection under long UV radiation (366 nm) |  |
|----------------|---|------------------|--|--|
|                | No of spots                                 | Rf               | No of spots                                | Rf   |
| <b>Extract</b> | 3   | 0.18, 0.23, 0.37 | 8  | 0.18, 0.23, 0.30, 0.37, 0.48, 0.56, 0.86, 0.95 |

Visualization<sup>[11]</sup>

Spray the air dried plate with Anisaldehyde Sulphuric acid reagent and the plate was heated at 110°C for 5 min. Rf value and colour of the band were noted.

Evaluation<sup>[12]</sup>

A band (Rf- 0.37) corresponding to *Costunolide* is visible in test solution track. As per quality standards of Indian Medicinal plants Vol-4, this Rf. Value is indicative of presence of *Costunolide* in this sample.

## CONCLUSION

A band (Rf.- 0.37) corresponding to *Costunolide* is visible in test solution track. As per standards this Rf. value is indicative of presence of *Costunolide* in this sample. Analytical and physicochemical profiles for standardization of medicinal plants is need of hour. *Costunolide* can be marker compound for the identification of *Kushtha* (*Saussurea lappa*. – C.B.Clarke.)

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