Quality control and phytochemical validation of *Saussurea lappa* (Costus/Qust)

Ayshah Hashimi¹, Akhtar Siddiqui¹, Yasmeen Ahmed¹, Mantasha Binth Siraj¹, Rizwana Khatoon²

¹Department of Moalajat, School of Unani Medical Education and Research, Jamia Hamdard, New Delhi, India, ²Department of Ilmul Advia, School of Unani Medical Education and Research, Jamia Hamdard, New Delhi, India

**Abstract**

**Introduction:** *Saussurea lappa*, traditionally known as *Qust* (*Costus*), is a perennial effective root, globally distributed across Himalayan region and has been extensively used for treating a variety of ailments for its antiulcer, anticonvulsant, anticancer, hepatoprotective, antiarthritic, and antiviral activities.

**Materials and Methods:** Organoleptic, physicochemical, phytochemical analysis, and chromatography of *S. lappa* are done as per the WHO guidelines for standardization of the herbal drug.

**Results:** This research resulted the physiochemical parameters such as moisture content, ash value as 7.46 ± 0.63, 6.33 ± 0.44 (total ash value), 2.33 ± 0.33 (acid insoluble), and 4 ± 0.28 (water soluble), respectively. Water extract contains the highest value (17.68%) of successive extraction. The extract shows four spots of different color in thin-layer chromatography.

**Discussion and Conclusion:** The results of preliminary phytochemistry profile of *S. lappa* (*Qust*) are actually useful in validating and determining the purity of the drug for the identification and documentation, which may be useful to pharmaceutical industries for the quality control of the commercial samples and also these characters will aid future investigators in their pharmacological analysis of this drug to develop them as a medicine.

**Key words:** Quality control, *Qust*, *Saussurea lappa*, standardization

**INTRODUCTION**

Plants have been a source of natural remedial agents since life came into existence. Herbs were also used in pre-Hippocratic period. Due to various biotic and therapeutic applications of active ingredients, herbal medicine is gaining importance these days and is foundation for revolution in drug discovery. Bioactive agents obtained from various herbal drugs are irreplaceable in the management of many intractable diseases and one such drug is *Saussurea lappa* (*Qust*), one of the best-known species of Asteraceae family, is a tall perennial herb possessing anthepatitis B⁴⁻⁵, antioxidant⁶⁻⁷, hepatoprotective⁸ and anticancerous⁹ activity. Morphologically its stem is stout and fibrous, root is long, firm with characteristics odor, leaves are lobate and stalked, flowers are dark purple, stalkless and are arranged at periphery. *S. lappa* comprises 300 different species in the world of which about 61 species exist in India⁸ and various biological active compounds are reported by different scientists.⁹ Numerous activities are tested, verified, and established through *in vitro* and *in vivo* methods that present a rational scientific approach to the traditional claims but before using the crude drug, standardization is very important for safety and efficacy of herbal products. Things to be kept in mind before using the crude drug is that – is the herb the one it should be? (For the identification of the drug), are there impurities, such as in the form of other herbs which should not be there? (For the purity of the drug) and is the content of active components within the definite limits? (Content or assay). Hence, quality control is needed to define shelf life, storage, distribution, chemical, physical, or biological properties which can be done by various parameters.
MATERIALS AND METHODS

Plant Material

Roots of *S. lappa* (*Qust*) were procured from “Nature & Nurture Healthcare Pvt. Ltd., 305, Vardhman City-2 Plaza, Asif Ali Road, New Delhi-110002.” Voucher specimen was deposited in the herbarium of the Department of Botany, School of Chemical and Life Sciences, Jamia Hamdard, New Delhi and was identified, authenticated, and certified as *Qust* (*S. lappa*). All standardization parameters were considered as per the WHO guidelines.[10]

Organoleptic Evaluation

It includes the evaluation of herbal drugs by size, shape, color, odor, and taste. It reveals morphological description of whole drugs.

Physiochemical Analysis

1. Foreign matter (FM)
2. Moisture content (M)
   About 5 g powdered *Qust* (*S. lappa*) was taken and spread out on Petri dish and was dried at 105°C for 6 h and weighed. M is calculated as:
   \[ M = \left( \frac{W_o - W_1}{W_o} \right) \times 100 \]
   Where 
   - \( M \) is moisture content.
   - \( W_o \) is weight of the sample.
   - \( W_1 \) is weight of empty Petri dish.
   - \( W_1 \) is weight obtained after successive drying.
   - \( W_1 \) is weight of dried sample (\( W_2 - W_1 \)).
3. Ash value
   About 5 g powdered *Qust* (*S. lappa*) was taken in a crucible and was ignited by gradually increasing the temperature up to 500–600°C until it turned ash, indicating the absence of carbon. Determination of ash value
   - Total ash value
     \[ \% \text{ Ash} = \frac{W_{Ash}}{W_{Dry}} \times 100 \]
     \( W_{Ash} \) is weight of the ash sample.
     \( W_{Dry} \) is weight of dried sample.
   - Acid-insoluble ash content
     \[ \% \text{ of acid-insoluble Ash} = \frac{W_{HCl}}{W_{Dry}} \times 100 \]
     \( W_{HCl} \) is weight of HCl insoluble ash.
     \( W_{Dry} \) is weight of dried sample.
   - Water soluble ash.
     \[ \% \text{ of water soluble Ash} = \frac{W_{H2O}}{W_{Dry}} \times 100 \]
     \( W_{H2O} \) is weight of water-soluble ash.
     \( W_{Dry} \) is weight of dried sample.
4. pH of 1% and 10% solution
   About 5 g and 10 g *Qust* was dissolved in 100 ml of distilled water separately, filtered and pH were measured.
5. Successive extractive value
   *Qust* (*S. lappa*) sample (25 g) was subjected to extraction with different solvents (petroleum ether, chloroform, methanol, and lastly water) through Soxhlet apparatus for 8 h at 40°C. All the extract obtained was evaporated to dryness and their constant extractive values were recorded.
6. Fluorescence analysis
   The powdered drug was subjected to different chemicals and then the color change was observed by ultraviolet spectrophotometer under daylight, 254 nm and 360 nm.
7. Phytochemical analysis (qualitative chemical test)
   The aqueous extract of *S. lappa* was subjected to preliminary phytochemical screening using standard screening method with different reagents as mentioned in Table 1
   - Preparation of aqueous extract
     Accurately weighed air-dried powdered drug (5 g) was placed in a glass-stoppered conical flask and then 100 ml water was

| Phytochemical components | Tests | 
|--------------------------|-------|
| Alkaloid                 | Dragendorff reagent+stock solution (1 ml)→reddish-brown color |
|                          | Hager’s reagent+stock solution (1 ml)→yellow ppt. |
|                          | Mayer’s reagent+stock solution (1 ml)→creamy ppt. |
| Carbohydrate             | Fehling’s solution (A+B)+stock solution (1 ml)→red color |
|                          | Benedict’s reagent+stock solution (1 ml)→boil→red color |
| Protein                  | Millon’s reagent (2 ml)+stock solution (1 ml)→boil→reddish-brown color or ppt |
|                          | Ninhydrin reagent (0.2%)+stock solution (1 ml)→violet color |
| Tannins                  | FeCl₃ (5%)+stock solution (1 ml)→green/blue-green color |
| Saponins                 | Foam test-water+stock solution (1 ml)→shake for 15 min→foamy layer on the top of the test tube |
| Flavonoids               | Stock solution (1 ml)+few drops NaOH→yellow color+dil. acid→colorless solution |
| Glycosides               | Liebermann’s test-2 ml acetic acid+2 ml chloroform+stock solution (1 ml)→cooled+H₂SO₄→green color |
| Phenolic compound        | Ferric chloride test-stock solution+FeCl₃→green/blue color |
|                          | Lead acetate test stock solution (2 ml)+2 ml of NaOH (10%)→boil→lead acetate |
|                          | Pb(C₂H₃O₂)₂→black/brown ppt |
added and weighed, including the flask. The solution was stirred well and then allowed to stand. After an hour, the solution was gently boiled by attaching reflux condenser for 1 h. The solution was left to cool down and then filtered rapidly by dry filter paper and transferred to water bath in flat bottomed Petri dish to evaporate to dryness. Further dried at 105°C for 6 h and cooled in a desiccator for 30 min and weighed without delay.

**Chromatography**

**Thin-layer chromatography (TLC)**

TLC assay was conducted on aqueous extract of *S. lappa* using toluene: ethyl acetate: formic acid: methanol (4:3:0.5:1) as mobile phase. Sulfuric acid reagent was used as detecting agent. Color, number, and R_f values of spots were noted.

## RESULTS

**Organoleptic Evaluation**

Dried sample of the drug comprises variable size (2–5 cm long and 0.5–1.5 cm thick) of pieces of root that is fusiform to cylindrical in shape and has collapsed center, seldom ridged and possess short, and horny fractures [Table 2].

**Physiochemical Analysis**

**Foreign matter**

Foreign Matter in *Saussurea lappa* was 2.42 (1%) which exhibits that the drug was least adulterated [Table 3 and Figure 1].

**M_c**

The M_c of roots of *S. lappa* was found to be 7.46%.

**Ash value**

a. Total ash value
   - Weight of sample drug = 2 g
   - Mean of total ash value = 6.33%.

b. Acid-insoluble ash content
   - Weight of sample drug = 2 g
   - Mean of acid-insoluble ash value = 2.33%.
Table 3: FM in *Saussurea lappa*

| Drug                  | Wt. of drug (g) A | Wt. of drug after removal of FM (g) B | Wt. of FM (g) A-B | Mean±standard error of the mean |
|-----------------------|-------------------|--------------------------------------|-------------------|---------------------------------|
| *Qust* (*Saussurea lappa*) | 250               | 248.13                               | 1.87              | 2.42±0.36                       |
|                       | 250               | 247.7                                | 2.3               |                                 |
|                       | 250               | 246.9                                | 3.1               |                                 |

FM: Foreign matter

Table 4: Moisture content in *Saussurea lappa*

| Wt. of drug W<sub>o</sub> (g) | Wt. of sample with Petri dish (g) | Wt. of sample after drying (g) | Loss on drying (g) | M<sub>o</sub> (%) (W<sub>2</sub>/W<sub>o</sub>)<sub>o</sub>×100 |
|------------------------------|----------------------------------|--------------------------------|-------------------|---------------------------------|
| 5                            | 145.39                           | 144.96                          | 0.43              | 8.6                             |
| 5                            | 156.23                           | 155.91                          | 0.32              | 6.4                             |
| 5                            | 146.45                           | 146.08                          | 0.37              | 7.4                             |
| Mean                         |                                  |                                 |                   | 7.46±0.63                       |

Table 5: Total ash value

| Drug                    | Wt. of crucible (g) | Wt. of crucible with drug (g) | Wt. of ash+crucible (g) | Wt. of ash sample (g) W<sub>Ash</sub> | Total ash (%) |
|-------------------------|---------------------|-------------------------------|-------------------------|--------------------------------------|----------------|
| *Qust* (*Saussurea lappa*) | 31.22               | 33.22                         | 31.35                   | 0.13                                 | 6.5            |
|                         | 32.34               | 34.34                         | 32.45                   | 0.11                                 | 5.5            |
|                         | 35.21               | 37.21                         | 35.35                   | 0.14                                 | 7              |
| Mean                    |                     |                               |                         |                                     | 6.33±0.44      |

Table 6: Acid-insoluble ash value

| Drug                    | Wt. of crucible (g) | Wt. of crucible with drug (g) | Wt. of ash+crucible (g) | Wt. of HCl insoluble ash+crucible | Wt. of HCl insoluble ash (g) W<sub>HCl</sub> | Total ash (%) |
|-------------------------|---------------------|-------------------------------|-------------------------|-----------------------------------|---------------------------------------------|----------------|
| *Qust* (*Saussurea lappa*) | 33.69               | 35.71                         | 33.83                   | 33.73                             | 0.04                                        | 2              |
|                         | 32.32               | 34.32                         | 32.54                   | 32.38                             | 0.06                                        | 3              |
|                         | 34.45               | 36.40                         | 34.79                   | 34.49                             | 0.04                                        | 2              |
| Mean                    |                     |                               |                         |                                    | 2.33±0.33                                    |                |

Table 7: Water-soluble ash value

| Drug                    | Wt. of crucible (g) | Wt. of crucible with drug (g) | Wt. of ash+crucible (g) | Wt. of water-soluble ash+crucible | Wt. of water-soluble ash (g) W<sub>H2O</sub> | Total ash (%) |
|-------------------------|---------------------|-------------------------------|-------------------------|-----------------------------------|---------------------------------------------|----------------|
| *Qust* (*Saussurea lappa*) | 31.22               | 33.22                         | 31.36                   | 31.29                             | 0.07                                        | 3.5            |
|                         | 32.32               | 34.32                         | 32.54                   | 32.4                              | 0.08                                        | 4              |
|                         | 33.69               | 35.69                         | 33.99                   | 33.78                             | 0.09                                        | 4.5            |
| Mean                    |                     |                               |                         |                                    | 4±0.28                                      |                |

Table 8: pH of solution

| Drug                    | pH of 1% solution | pH of 10% solution             |
|-------------------------|-------------------|--------------------------------|
| *Qust* (*Saussurea lappa*) | 6.61              | 6.50                           |

c. Water-soluble ash
Weight of sample drug = 2 g
Mean of water-soluble ash value = 4% [Table 4].
High inorganic substances present in the herbal drugs are explained by ash values. So, the salts of Na + and Ca 2+
Table 9: Successive extractive value of Qust (Saussurea lappa)

| Solvents (500 ml) | Wt. of sample (g) | Wt. of Petri dish (g) | Wt. of extract with Petri dish (g) | Wt. of extract (g) | % of extract |
|-------------------|-------------------|-----------------------|------------------------------------|-------------------|-------------|
| Pet. ether        | 25                | 44.63                 | 45.13                              | 0.50              | 2           |
| Chloroform        | 41.56             | 45.46                 | 3.90                               | 15.6              |
| Methanol          | 43.21             | 45.36                 | 2.15                               | 8.6               |
| Water             | 45.27             | 49.69                 | 4.42                               | 17.68             |

Table 10: Fluorescence analysis under daylight, 254 nm and 360 nm

| Reagents          | Daylight          | 254 nm | 366 nm |
|-------------------|-------------------|--------|--------|
| Conc. HCl         | Dark red          | Light red | Dark red |
| Conc. HNO₃        | Red               | Light brown | Reddish-brown |
| Ethyl acetate     | Light brown       | Dark brown | Brown |
| Acetone           | Yellow            | Straw | Light yellow |
| Chloroform        | Yellowish-green   | Light green | Greenish-yellow |
| Petroleum ether   | Yellowish-brown   | Yellowish-brown | Brown |
| Methanol          | Light brown       | Dark brown | Dark brown |
| Conc. H₂SO₄      | Dark brown        | Reddish-brown | Brown |
| Glacial acetic acid | Dark orange     | Yellowish red | Light orange |
| Water             | Yellowish-brown   | Yellow | Yellowish-brown |

Table 11: Phytochemical screening

| Constituents     | Result |
|------------------|--------|
| Alkaloid         | +      |
| Carbohydrates    | +      |
| Glycosides       | +      |
| Tannins          | +      |
| Phenolic compounds | +   |
| Flavonoids       | +      |
| Proteins         | −      |
| Saponins         | +      |

Table 12: Thin-layer chromatography profile of aqueous extract of Qust (Saussurea lappa)

| Drug             | Solvent system                         | Rₜ value | No. of spot |
|------------------|----------------------------------------|----------|-------------|
| Qust (Saussurea lappa) | Toluene:ethyl acetate:formic acid:methanol (4:3:0.5:1) | 0.83 (Blue) | 04          |
|                  |                                        | 0.71 (Green) |             |
|                  |                                        | 0.63 (Pink)  |             |
|                  |                                        | 0.50 (Green)  |             |

pH of 1% and 10% solution

pH of 1% solution was 6.61 while pH of 10% solution was 6.5 [Table 8].

Successive extractive value

The amount of ingredients presents in a drug separate with solvents from a given quantity of medicinal plant material showed the extractive values. Different solvents such as petroleum ether, chloroform, methanol, water was used for successive extraction of test drug by using Soxhlet apparatus. The values of successive extraction of petroleum ether, chloroform, methanol, water was measured as 2%, 15.6%, 8.6% and 17.68% respectively [Table 9 and Figure 3].

Fluorescence analysis

Different chemical regents such as Conc. HCl, Conc. HNO₃, Conc. H₂SO₄, chloroform, glacial acetic acid, etc. were used for fluorescence analysis and were gazed under daylight, at 254 nm and 360 nm and presented different colours [Table 10].

Phytochemical analysis

Preliminary phytochemical screening of Saussurea lappa (Qust) was studied on aqueous extract and lots of chemical tests has been performed for different phytochemical components (qualitative test) such as phenols, carbohydrates and proteins. Alkaloids, phenolic compounds, flavonoids,
glycosides, tannins and saponins was present while Ninhydrin Test for amino acids was negative [Table 11].

**Chromatography**

**TLC**

There were four spots of different color, i.e., blue, green, pink, and green appearing at Rf 0.83, 0.71, 0.63, and 0.50, respectively [Table 12 and Figure 4].

**CONCLUSION**

Uniformity in the quality of plant material is necessary to prevent variation in superiority, safety, and efficacy of the same formulation manufactured in different areas. The present research article evaluates the quality of the sample and validates the phytochemical screening to understand its uses and approves clinical application described in classical Unani literature. In this study, introductory phytochemical screening of the aqueous extracts shows the presence of various phytoconstituents, i.e., various alkaloids, flavonoids, saponins, glycosides, phenolic compounds, etc. These bioactive agents serve as anti-inflammatory, hepatoprotective, antioxidant, and anticancerous agents. These properties may be the reason for its ethnomedical use in several diseases defined in classical literature. It also reveals its great scope for future research as it has some very interesting phytochemicals; moreover, isolation and purification of pure compounds should be carried out.

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