ORIGINAL ARTICLE

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

Ayshah Hashimi[1], Akhtar Siddiqui[1], Yasmeen Ahmed[1], Mantasha Binth Siraj[1], Rizwana Khatoon[2]

[1]Department of Moalajat, School of Unani Medical Education and Research, Jamia Hamdard, New Delhi, India, [2]Department 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 antihepatitis B[1,2] antioxidant[3,4] hepatoprotective[5] and anticancerous[6,7] 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 India[8] 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.

Address for correspondence:
Dr. Mantasha Binth Siraj,
Department of Moalajat, School of Unani Medical Education and Research, Jamia Hamdard,
New Delhi - 110 062, India. Phone: +91-9899250255.
E-mail: mantashasiraj@gmail.com

Received: 28-06-2019
Revised: 11-11-2019
Accepted: 23-11-2019

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_c)
   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_c is calculated as:
   $$M_c=(W_o-W_3/W_o)\times 100$$
   Where M_c is moisture content
   W_o is weight of the sample
   W_1 is weight of empty Petri dish is weight obtained after successive drying
   W_3 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
     % Ash=$W_{Ash}/W_{Dry}\times 100$
     W_{Ash} is weight of the ash sample
     W_{Dry} is weight of dried sample.
   - Acid-insoluble ash content
     % of acid-insoluble Ash=$W_{HCl}/W_{Dry}\times 100$
     W_{HCl} is weight of HCl insoluble ash
     W_{Dry} is weight of dried sample.
   - Water soluble ash.
     % of water soluble Ash=$W_{H2O}/W_{Dry}\times 100$
     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

Table 1: Phytochemical analysis using standard screening method

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	$FeCl_3$ (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+H_2SO_4→green color
Phenolic compound	Ferric chloride test-stock solution+$FeCl_3$→green/blue color
	Lead acetate test stock solution (2 ml)+2 ml of NaOH (10%)→boil→+lead acetate $Pb(C_2H_3O_2)_2$→black/brown ppt

Table 2: Morphological description

Characteristics	Physiognomy
Color	Muddy greyish to light brown
Odor	Strong, aromatic, and penetrating
Taste	Bitter
Shape	Fusiform to cylindrical, twisted
Consistency	Solid
Size	7–14 cm long, 1–5 cm diameter

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%.

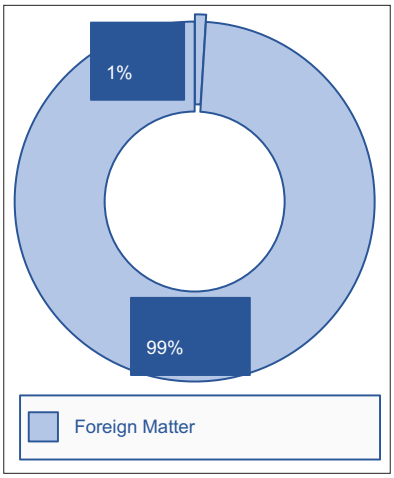

Figure 1: Percentage of foreign matter

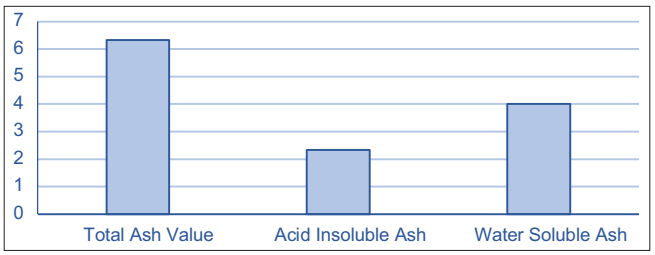

Figure 2: Ash values

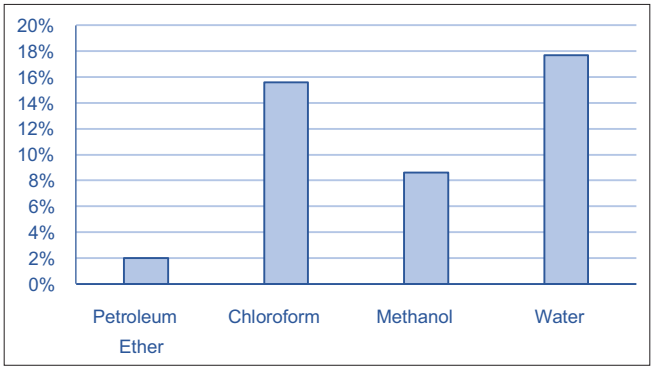

Figure 3: Successive extractive values

Figure 4: Thin-layer chromatography image of *Saussurea lappa*

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_o (g)	Wt. of sample with Petri dish (g)	Wt. of sample after drying (g)	Loss on drying (g)	M_c (%) $(W_2/W_o)\times100$
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_{Ash}	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	Wt. of ash+crucible	Wt. of HCl insoluble ash+crucible	Wt. of HCl insoluble ash (g) W_{HCl}	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	Wt. of ash+crucible	Wt. of water-soluble ash+crucible	Wt. of water-soluble ash (g) W_{H2O}	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. HNO_3	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. H_2SO_4	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	R_f value	No. of spot
Qust (*Saussurea lappa*)	Toluene:ethyl acetate:formic acid:methanol (4:3:0.5:1)	0.83 (Blue) 0.71 (Green) 0.63 (Pink) 0.50 (Green)	04

are responsible for the presence of ash content, these are not injurious. 6.33%, 4%, 2.33% are the values total ash, water soluble ash and acid insoluble ash respectively of dry weight of the drug [Tables 5-7 and Figure 2].

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. HNO_3, Conc. H_2SO_4, 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 R_f. 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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Source of Support: Nil. **Conflict of Interest:** None declared.