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ANALYTICAL STUDY OF HYDRAULIC EXTRACT OF *KUSHTHA* (*SASSUREA LAPPA* C.B. CLARKE.) ROOT

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ABSTRACT:

Background: *Sassurealappa* C.B. Clarke., -*Kushtha* (*Costus*) is an Ayurvedic annual herb of the eastern Himalayas (Kashmir) used particularly as an anti-inflammatory, anthelmintic, expectorant used in wide variety of Ayurvedic formulations. **Aims:** The study was carried out for standardization of aqueous extract of *Sassurealappa* C.B. Clarke. -*Kusta* (*Costus*). **Settings and Design:** 6 samples in 3 batches of aqueous extract of *Sassurea lappa* were carried out for Physical analysis and Analytical techniques like HPTLC fingerprinting and Assay of total alkaloids performed by Gravimetric Method. **Methods and Material:** *Sassurea lappa* roots from the local market were taken for standardization. Physico chemical, phyto chemical, organoleptic and Analytical parameters were carried out as per the guidelines of Ayurveda Pharmacopeia and WHO drug standardization methods. **Results and Conclusions:** Analysis shows values of Particle size through 40 mesh 99.08%, Loss on Drying 3.76%, pH 3.12, Water soluble Extractive 97.15%, Alcoholic soluble Extractive 84.65%, Total ash 5.35%, Acid insoluble ash 0.68 %, Bulk Density (gm/ml) 0.72 and Trapped density (gm/ml) 0.97. Heavy metal, Microbial and Organoleptic values are also within the prescribed limits. HPTLC graph shows peak value of the total height 77.3 and total area 1499.0. R_f (Retention fraction) value of Sesquiterpene lactones is 0.32. Assay of total alkaloids was 0.27%. Analysis of the extract shows values within the Ayurveda Pharmacopeia WHO guide lines. The HPTLC method was a simple, precise, specific, sensitive and accurate and used for routine quality control of raw material as well as formulation containing any of these compounds.

KEY WORDS: Ayurveda, Acid insoluble ash, Analytical Techniques, HPTLC fingerprinting, *Sassurea lappa*,

INTRODUCTION

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Ayurveda the Indian System of Medicine describes wide variety of formulations for most range of ailments using plant products. Plant and herbal materials are using widely in the developing and developed countries as a mainstream of medicine or as an alternative medicine.^{1, 2} Eighty of world population is dependent on herbal drugs and to enter into the global market it is vital to maintain its quality. The quality of herbal drugs is the sum of all factors which contribute directly or indirectly to the safety, effectiveness and acceptability of the product.³ Standardization of Herbal product is a big and an important issue. Analytical techniques like High Performance Thin Layer Chromatography (HPTLC) finger printing, physical analysis, pH, total ash, acid insoluble ash, bulk density, tapped density, heavy metals, assay of marker compound by HPLC (High Performance Liquid Chromatography) method has a pivotal role in quality control and standardization.^{4, 5} Standardization and

quality control of herbal as well as the Ayurvedic products is most essential for the acceptance on the modern parameters.⁶ Naturally derived herbal and botanical extracts will experience some of the fastest growth among the major nutraceutical ingredient groups, according to “World Nutraceutical Ingredients to 2015”.^{7, 8} *Sassurea lappa* (*Kuth*) native to the Himalayan Region is one of the most commercially viable species of this genus and is in high demand for treating many diseases like bronchial asthma, rheumatism, cholera, jaundice, leprosy, etc. Considering the high industrial demand for raw materials and the endangered status of *Sassurea*, adulteration is most possible.⁹ Standardized extracts believe that they represent a trend towards higher technological refinement will provide a more consistent, stronger and more effective product backed by chemical analysis to confirm the presence and ratio quantity of one or a number of characteristic plant constituents. Standardization of extract will assess the qualitative and quantitative of marker compound.^{11, 12} Ayurveda emphasizes the importance of standardization of medicinal herbs as well as the finished products on the basis of physical and chemical parameters like the shape, texture, smell of the useful part.

MATERIAL AND METHODS

METHODOLOGY

The physicochemical, organoleptic and spectrographic studies conducted at Laila impex, R &D division Vijayawada. Physico-chemical parameters of the *Sassurea lappa* were assessed as per guidelines of Indian system of Medicines and Homeopathy and Ayurveda Pharmacopeia.¹¹Total ash values, loss on drying, water soluble ash, acid insoluble ash, heavy metals, alcohol soluble extractive and water soluble extract values were determined.¹⁰

COLLECTION OF PLANT MATERIAL

Sassurea lappa roots are purchased for the local market Vijayawada and identified and authenticity by the pharmacognogist, Institute of applied Ayuveda Sciences, Pune. *Sassurealappa*, *C.b.Clarke* belonging to the family *Asteraceae* commonly known as the Costus root is a

perennial herb. Root is the useful part.^{13, 14, 15}

Authentication: The identity of the plant was confirmed by Dr. Murthy S N, Assistant Director incharge, National Institute for Basic Principles of Ayurveda, Pune. A voucher specimen of the sample (No.1) and raw drug has been identified, authenticity, and on the basis of Microscopic and Macroscopic Characters as per Quality standards of Indian Medical Plants, Indian Council of Medical Research, New Delhi(India), Vol 7 pp-204-207.

The water extracts of *Sassurea lappa* C .B. Clarke kept in a cool temperature in an airtight double foiled package for further studies. (Batch Number: L 10060517).

Organoleptic characters

Colour	Texture	Odour	Taste
Brown	Dry Powder	Aromatic	Bitter

PHYSICO CHEMICAL STUDIES:

Physico-chemical parameter of the *Saussurea lappa* was determined as per Guidelines of Indian system of Medicines and Homeopathy and Ayurveda Pharmacopeia.¹⁰Total ash values, loss on

drying, water soluble ash, acid insoluble ash, heavy metals, alcohol soluble extractive and water soluble extract values were determined.¹⁰

MICROBIAL SCREENING

Microbial screening was carried out for the safe use of the individual plant extract and checked for total aerobic count, total yeast and mould count.¹⁰

PREPARATION OF EXTRACTS

The fresh root sample of *Saussurea lappa* was air dried and powered. The dried powder of the root was treated for extraction by hot water for 6 hours. The process was repeated twice. The pooled extract was concentrated and dried under vacuum, until it forms to dry flakes. Dry flakes pulverized by multimill/

micropulviniser and sieved on shifter and packed.

RESULTS

Six Samples in 3 batches of *Saussurea lappa* water extract were studied for its characteristics and physico-chemical standards like total ash, water soluble ash, acid insoluble ash, water soluble extractive, ethanol soluble extractive and moisture content are shown on table .1

Table1. Physicochemical standards

S.No.	Physico chemical parameter	Value of Water extract of <i>Saussurea lappa clarke</i> .
1.	Particle size through 40 mesh	99.08%
2.	Loss on drying	3.76%
3.	Water soluble extractive	97.15%
4.	Alcoholic soluble extractive	84.65%
	pH	3.12
5.	Total ash	5.35%
6.	Acid insoluble ash	0.68 %
7.	Bulk density(gm/ml)	0.72
8.	Tapped density (gm/ml)	0.97

Table 2.Limits of Heavy Metals

S.No.	Heavy metal	Values in Water extract of <i>Saussurea lappa</i>
1	Arsenic	< 2ppm
2.	Lead	< 5ppm
3	Cadmium	< 1ppm

Analysis reveals a minor presence of some of heavy metals but the sample does not

exceed the limits given according to WHO guidelines.^{10, 11.}

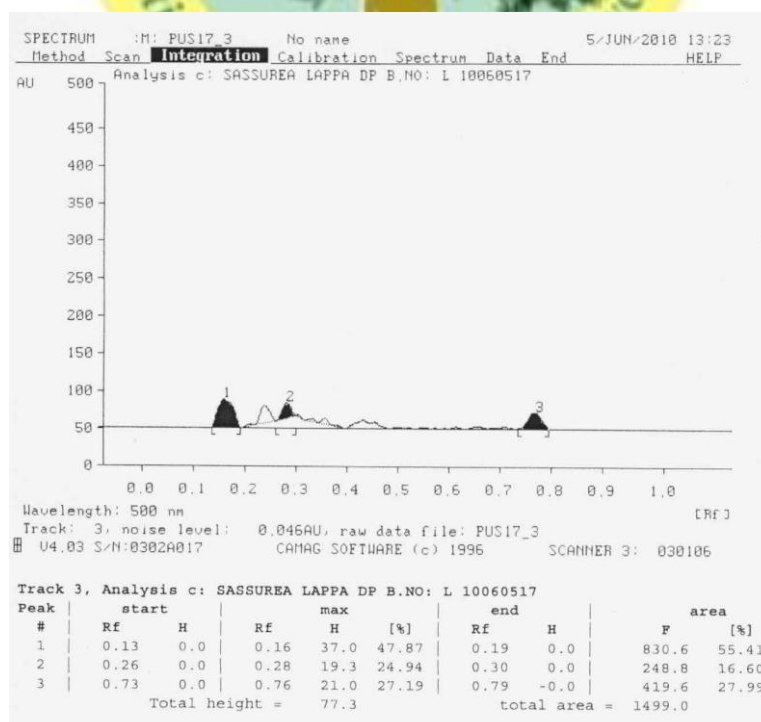
Table 3. Microbial Screening of the Extract

S.No.	Microbial type	Values in water extract of <i>Saussurea lappa</i>
1	Total plate count	<1000 CFu/gm
2.	Yeast , Moulds	< 10 CFu/gm
3.	<i>Enterobacteriae</i>	Absent
4.	<i>E.coli</i>	Absent
5.	<i>Salmonellae</i>	Absent
6.	<i>S.aureus</i>	Absent
7.	<i>Staphylococcus aureus</i>	Absent
8.	<i>Pseudomonas aeruginosa</i>	Absent

Microbial count is within the WHO standards and safe for the formulation.^{11, 12.}

Spectroscopic screening: Spectroscopic screening performed at Laila Impex, R & D Division, Vijayawada. Thin layer Chromatography/High performance Thin Layer Chromatography method for identification *Saussurea lappa* Extract Dry Powder (DP B.NO: L 10060517) Carry out

using silica gel 60 F₂₅₄ precoated foil plate with layer thickness 0.2 mm (Merk, Germany). Evaluate the chromatoplate in W 500nm. Visualization and Scan at 500nm by using LINOMAR IV (CAMA G, Sonnemattstise, 17, Switzerland).

Figure: HPTLC *Saussurea lappa* Extract Dry Powder

Inference: HPTLC of *Sassurea* water extract produced three peaks between RF: 0.10-0.80 with total height and total area of 77.3 and 1499.0 respectively. (Table 4)

Table: 4. High Performance Thin Layer Chromatography

Peak #	Start		max			end		area	
	Rf	H	Rf	H	%	Rf	H	F	%
1	0.13	0.0	0.16	37.0	47.87	0.19	0.0	830.6	55.41
2	0.26	0.0	0.28	19.3	24.94	0.30	0.0	248.6	16.60
3	0.73	0.0	0.76	21.0	27.19	0.79	0.0	419.6	27.99

Assay of total alkaloids in *Sassurea* extract performed by Gravimetric Method and was 0.27%.

DISCUSSION

The main aim of the paper is to standardize the aqueous extract rather than the Hydro alcoholic of methanol extracted *Sassurea* (*Chengalva Koshtu*) purchased from the local market---.An attempt had made for study of the water extract of *Kushtha* (*Sassurea lappa*) root for organoleptic characters and subjected to physicochemical analysis. Simple and available techniques like High Performance Thin Layer Chromatography finger printing for further studies and utility. Ayurveda pharmacopeia of India and other articles on standardization of *Sassurea* mentions only crude drug powder.^[11,17] Physico chemical parameters shows the values of particle size through 40 mesh (99.08%),pH (3.12), loss on drying (3.76%), water soluble extractive (97.15%), total ash content (5.35%), acid

insoluble ash, (0.68 %), bulk density value (0.72 gm/ml), tapped density value (0.97 gm/ml), heavy metals Arsenic (< 2ppm), Lead(< 5ppm)and Cadmium (< 1ppm). Assay of total alkaloids estimated by Gravimetric method was 0.27%.HPTLC of *Sassurea lappa* water extract revealed three peaks between Retention fraction (Rf) of 0.10-0.80 with total height and total area of 77.3 and 1499.0 respectively. Microbial screening test results of *Sassurea lappa* water extract was within the prescribed limits of Ayurveda Pharmacopoeia and was of standard quality even for the water extract of *Sassurea lappa*.^{5, 11, 12}. Simple aqueous extract had taken and *Sassurea* was not supposed to percolate with highly toxic and strong solvents like hexane, benzene, and methyl chloride acetate. Analysis reports will reveal a minute quantity of toxic solvents if percolated and extracted with other solvents rather than water. Efficacy, qualitative, quantification and percentage of active compound in aqueous

extract of *Sassurea* may study for the future clinical studies. Even though a number of species of *Sassurea* are available in the market genuinely of *Sassurea lappa* can be standardized by using the marker compound identification. Adulteration of *Sassurea lappa*, with organic and inorganic compounds and *Arctium lappa*,¹⁷ belonging to the same family, is one such plant that has often been found to be present in the market samples of 'Kuth' wild variety of *Sassurea* genus, can be identified by HPTLC fingerprinting. Genuinely of *Sassurea lappa* can

be standardized by using the marker compound identification. The HPTLC finger print of the drug is also useful to verify the quality and determine the same drug in compound formulations.⁵ Microbial and heavy metals values are within the limits of Ayurveda Pharmacopeia and WHO guidelines.^{11, 12, 15, 16} The results of the *Sassurea lappa* water extract shows the prescribed limits of the Pharmacopoeia and are of standard quality and the microbial limits and the heavy metals were in safe limits.

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