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Research Article

Formulation, Evaluation and Validation of Newly Formulated Laportea Arishta

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ABSTRACT

Laportea Arishtas are self generated herbal fermentations made with decoctions of *Laportea interrupta* leaves. The improvement in the extraction of drug molecule from the herb, improvement in drug delivery into the human body sites and enhancing therapeutic properties of the herb are the main reason for selecting this herb. Physico chemical analysis was performed for the crude drug for assuring its authenticity. The formulated arishta were subjected to organoleptic-physico-chemical analysis, qualitative and quantitative analysis, antioxidant studies along with a new analytical method validation. The quantitative estimations were performed by determining the alcoholic, phenolic, flavanoid contents along with reducing sugar determination. In vitro antioxidant activity was performed by FTC, FRAP and TBA method. Validation experiments of new *Laportea* arishta were performed to demonstrate linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ as per ICH guidelines.

Key words: Laportea interrupta, Arishtas, Antioxidant**,** Validation.

INTRODUCTION

Now a day's majority of people were fully dependant on ayurvedic drugs. *Laportea interrupta (L.) Chew of family urticaceae* having common name Hawaii wood nettle, is a herb having many traditional uses. *Arishta* and *Asava* have been used as medicines for over 3000 years to treat various disorders and are also taken as appetizers and stimulants. For longer periods higher doses of these drugs can be used. It is of sweet taste, medicinal value, easy availability etc.

Arishta and *Asava* occupy an important place in the ayurvedic pharmaceutical industry for its manufacture and sale. Asavas and arishta are medicinal preparations made by soaking the drugs, either in coarse powder form or in the form of decoction (Kashaya), in a solution of sugar or jaggery. It is for a specified period of time, results in fermentation generating alcohol. The extractions of the active principles contained in the drugs were facilitated. The alcohol, so generated, also serves as a preservative. Many of the phytochemical have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases. Ayurvedic medical system is based on the system of prevention which means that people are often treated before the symptoms of the illness become evident.

Antioxidants acts in several ways including scavenging reactive oxygen or nitrogen species, inhibiting ROS formation etc. The most common cellular free radicals are superoxide radical, nitric oxide and hydroxyl radical. Phenolic compounds are important secondary metabolites present in plants and are also responsible for their antioxidant actions which are under the category of polyphenols. Flavanoid are natural products widely distributed in plant kingdom. Flavanoid are powerful antioxidants and are described as free-radical scavengers against free radicals. Validation results in a tabular summation of the characteristics applicable to identification control of impurities and assay procedures.

Experimental, Materials and methods

The leaves of *Laportea interrupta (L.) Chew of family urticaceae* were collected from Kottakkal, Kerala during the month of august 2012 and authenticated by A.K. Pradeep, Ph.D, Herbarium Curator, Department of Botany, Calicut University and has been stored in university herbarium as herbarial specimen No:107889.

Chemicals Used

Ethyl acetate, Methanol, Petroleum ether, Chloroform, Distilled water, Hydrochloric acid, Sodium hydroxide, Glacial acetic acid, Sulphuric acid, Ether, Ethanol, Methanol, Potassium hydroxide, Sodium carbonate, Gallic acid and Quercetin were of analytical grade from and purchased from Mark chemicals, India. Fehling's reagent A and B, Benedict's reagent, Molisch reagent, Iodine, Millions reagent, Ninhydrin reagent, Folin ciocalteau reagent, Ferric chloride, Ferrous sulphate, Lead acetate, Magnesium sulphate, Mayer's reagent, Wagner's reagent, Potassium dichromate, Linoleic acid, Ammonium thiocyanate, Disodium hydrogen phosphate, Potassium dihydrogen phosphate, Aqueous trichloroaceticacid, Thiobarbituric acid, TPTZ, Ammonium acetate, Ammonia were of analytical grade from and purchased from MEDILISE CHEMICALS, Kannur,and NICE CHEMICALS, Kochi.Honey, sugar, jaggery, earthen pot and lid, ribbon cloth, crucible from the local market of Calicut.

Instruments

Soxhlet apparatus, UV instrument (ELICO SL 164 UV-Visible spectrophotometer)

Collection of Plant Materials

The leaves of *Laportea interrupta* were collected early morning and washed well to remove any adhering foreign

particles and soil materials. The washed plant leaves were dried under shade and coarsely powdered.

Physicochemical Analysis of Crude Drug (Table 1)

Physicochemical analysis of crude drug was performed for identifying the quality and purity of crude drug[1],[6].

Total Ash Value

Silica crucible was heated and allowed to cool and weighed. About 1 gm dried powder was taken and ignited to constant weight.

Table 1 Physico-Chemical Analysis of Crude Drug *Laportea Interrupta*

Test	Result
Rodent Contamination	Absent
Foreign Organic Matter	0.54%v
Insect Infestation	Absent
Total Ash	0.16(g)
Acid Insoluble Ash	0.33(g)
Water Insoluble Ash	0.11(g)
Sulphated Ash	0.36(g)
Moisture Content	0.235(g)

Acid Insoluble Ash

The total ash was boiled with 25ml of hydrochloric acid for 5 min; the insoluble matter was collected in ash less filter paper, washed with hot water, ignited, cooled and weighed.

Water Insoluble Ash

The total ash was boiled for 5 min with 25 ml water, the insoluble matter was collected in ash less filter paper, washed with hot water ignited, cooled and weighed.

Sulphated Ash

2gm of powdered drug was taken in silica crucible and 3 ml of sulphuric acid was added and incinerated until free from carbon.

Moisture Content

10 gm sample was taken in tarred china dish and dried in oven at 100° C, cooled and weighed, recorded and proceeded till obtaining a constant weight.

Extraction of Plant Material

The plant material was dried in the shade, then coarsely powdered and extracted in Soxhlet Apparatus using various solvents according to their polarity[2].

Ethyl Acetate Extract

The coarse leaf powder of Laportea interrupta was taken about 20gm and extracted with 250ml of ethyl acetate (70-80°c) by continuous hot percolation using Soxhlet apparatus. After completion of extraction, it was filtered and the solvent was removed by distillation under the reduced pressure. The extract was stored in desiccators. The % yield obtained was 2.78%

Petroleum Ether Extract

The coarse leaf powder of Laportea interrupta was taken about 20gm extracted with 250ml of Petroleum Ether (60-80°c) by continuous hot percolation using Soxhlet apparatus. After completion of extraction, it was filtered and the solvent was removed by distillation under the reduced pressure. The extract was stored in a dessicator. The % yield obtained was 1.52%

Chloroform Extract

The coarse leaf powder of Laportea interrupta was taken about 20gm, extracted with 250ml of Chloroform (50.5-61.5°c) by continuous hot percolation using Soxhlet apparatus. After completion of extraction, it was filtered and the solvent was removed by distillation under the reduced pressure. The extract was stored in a dessicator. The % yield obtained was 1.95%.

Methanol Extract

The coarse leaf powder of Laportea interrupta was taken about 20gm extracted with 250ml of methanol (60-70°c) by continuous hot percolation using Soxhlet apparatus. After completion of extraction, it was filtered and the solvent was removed by distillation under the reduced pressure. The extract was stored in a dessicator. The % yield obtained was 1.90%

Aqueous Extract

The coarse leaf powder of Laportea interrupta was taken about 20gm extracted with 250ml of Distilled water (0.25%) by continuous hot percolation using Soxhlet apparatus. After completion of extraction, it was filtered and the solvent was removed by distillation under the reduced pressure. The extract was stored in a dessicator. The % yield obtained was 1.26%.

Quantitative Phytochemical Analysis of Ethyl Acetate Extract

For quantitative analysis ethyl acetate extract is used because it found to have high yield of phenol and flavanoid content (Table: 2)

Table 2 Qualitative Phytochemical Analysis

Constituents	Ethyl acetate	Pet.ether	Chloroform	Methanol	water
Proteins	-	-	-	-	-
Carbohydrate	+	-	-	+	+
Phenols and Tannins	+	-	-	+	+
Flavanoids	+	-	-	+	-
Saponins	-	-	-	-	-
Glycosides	+	-	-	+	+
Steroids	+	+	-	+	-
Terpenoids	-	+	+	-	-
Alkaloids	+	-	-	+	-

(-): TEST NEGATIVE, (+): TEST POSITIVE

Total Phenolic Content

Total phenol content of samples was measured as Gallic acid equivalents.

Preparation of Standard Solution

Gallic acid was taken about 1gm and dissolved in 1000ml of distilled water, which was the stock solution (1mg/ml or 1000µg/ml). From the stock solution 1ml, 2ml, 3ml, 4ml, 5ml (10,20,30,40,50 µg/ml) were taken to 100ml standard flask and made with distilled water. 5ml of folin ciocalteau reagent and 4ml of 1M sodium carbonate were added to 1ml of standard serial dilution, then incubated at dark for 30 minutes and then absorbance were measured at 765nm in UV visible spectrophotometer (SCHIMADZU UV-Visible spectrophotometer) and a linear graph was plotted using the absorbance of standard serial dilutions and found out the phenol content.

Preparation of Sample Solution

5ml of folin ciocalteau reagent and 4ml of 1M sodium carbonate were added to 0.1ml of extract, then incubated at dark for 30 minutes and then absorbance was measured at 765nm in UV visible spectrophotometer.

Total Flavanoid Content

Total Flavanoid content of samples was measured as Quercetin equivalents.

Preparation of Standard Solution

Quercetin was taken about 1gm and dissolved in 1000ml of methanol, which was the stock solution (1mg/ml or 1000µg/ml). From the stock solution 1ml, 2ml, 3ml, 4ml, 5ml (100, 200, 300, 400, 500 µg/ml) were taken to 10ml standard flask and made with methanol.

Standard serial dilution absorbances were measured at 420nm in UV visible spectrophotometer and a linear graph was plotted using the absorbance of standard serial dilutions and found out the Flavanoid content.

Preparation of Sample Solution

Aluminium chloride colorimetric method was used for the determination of Flavanoid content. 0.1ml of extract was mixed with 3ml of methanol, 0.2ml of 10% aluminium chloride, 0.2ml of potassium acetate and 5.6ml of distilled water, then incubated at room temperature for 30 minutes and then absorbances were measured at 420nm in UV visible spectrophotometer.

Preparation of Formulation

Arishtas are self-generated herbal fermentations of traditional ayurvedic system. They are alcoholic medicaments prepared by allowing herbal decoctions to undergo fermentation with the addition of sugars[2,3]. (S.Sekar et al, 2008). The ethyl acetate extract giving high % yield was used for formulation preparation.

Process of preparation

The earthen pot sufficiently large and strong glazed exterior was tested for weak spots and cracks and similarly a lid was also chosen. Distilled water was boiled in it twice up to its rim and dried out, then wiped the pot and lid using clean dry cloth and smeared cow's ghee on the surface. The proportion of the different ingredients were 32 seers (1024 tolas were 1tolas=12 gm) of distilled water, boiled and added 1.25 seers dried plant leaf powder to make decoction, after cooling the decoction, filtered and obtained a clear solution. To this decoction jaggery was added 12.5 seers, honey 6.25 seers and sugar 6.25 seers, mixed well to form a uniform solution.

Inoculum

To the prepared earthen pot mixture was added up to its three by forth and then lid was closed and sealing was done by cleaned ribbon and clay was smeared tightly over the ribbon. Pot was buried in a pit made in the soil for 30 days, on 31st day the pot was taken out and mud smeared on it and ribbon removed carefully. The lid was opened carefully without allowing any dust into it and fermented solution then filtered through a muslin cloth previously washed and the clear arishta was then stored in previously washed and dried tightly stoppered glass bottles for further use.

Qualitative Phytochemical Analysis

All the extracts were subjected to qualitative tests for identification of phytochemical constituents present in it[4] (Table No: 3).

Test for Protein

Million's Test: Crude extract was mixed with 2ml millions reagent and heated, white precipitate appeared which turned red upon gentle heating indicate the presence of proteins.

Ninhydrin Test: Crude extract was boiled with 2ml of 0.2% solution of ninhydrin, violet color appeared indicate the presence of proteins.

Table 3 Quantitative Phytochemical Analysis of Ethyl Acetate Extract

Constituents	Ethyl Acetate extract
Total phenol content	38 ± 4 (mg/g)
Total Flavanoid Content	260 ± 10 (mg/g)

Test fir Carbohydrates

Fehling's Test: Equal volume of Fehling's A and B reagent were mixed together and 2ml of it was added to crude extract and heated gently, a brick red precipitate appeared at the bottom of test tube indicate the presence of carbohydrates.

Benedict's test: Crude extract was mixed with 2ml Benedict reagent and boiled, a reddish brown precipitate formed indicate the presence of carbohydrates.

Molisch's Test: Crude extract was mixed with 2ml molisch reagent and added 2ml concentrated sulphuric acid along the sides of test tube, appearance of a violet ring at the interphase indicate the presence of carbohydrates.

Iodine Test: Crude extract was mixed with 2ml iodine solution, a dark blue or purple indicate the presence of carbohydrates.

Test for Phenols and Tannins

Ferric Chloride Test: Crude extract was mixed with 2ml of 2% solution of ferric chloride, blue-green or black coloration indicates the presence of phenols ad tannins.

Lead Acetate Test: Crude extract was mixed with 2ml of 10% lead acetate solution; white coloration indicates the presence of phenols and tannins.

Test for Flavanoids

Shinoda Test: Crude extract was mixed with few fragments of magnesium ribbon and concentrated hydrochloric acid was added drop wise, pink scarlet color appeared after few minutes, indicates the presence of Flavanoid.

Alkaline Reagent Test: Crude extract was mixed with 2ml of 2% solution of sodium hydroxide, an intense yellow color formed which turned colorless on addition of few drops of dilute acid, indicates the presence of Flavanoid.

Test for Saponins: Crude extract was mixed with 5ml distilled water in a test tube and shaken vigorously, the formation of stable foam indicate the presence of saponin.

Test for Glycosides

Liebermann's Test: Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice and carefully added concentrated sulphuric acid, a color change from violet to blue indicate the presence of steroidal nucleus,

Salkowski's Test: Crude extract was mixed with 2ml of chloroform and then 2ml of concentrated sulphuric acid was added carefully and gently shaken, a reddish brown indicate the presence of steroidal nucleus.

Keller-Kilani Test: Crude extract was mixed with 2ml of glacial acetic acid and containing 1-2 drops of 2% solution of ferric chloride, the mixture was poured into another test tube containing 2ml of concentrated sulphuric acid, a brown ring at the interphase indicate the presence of cardiac glycoside.

Test for Steroids: Crude extract was mixed with 2ml of chloroform and concentrated sulphuric acid was added sidewise, a red color produced in the lower chloroform layer indicates the presence of steroids.

Mixing crude extract with 2ml chloroform, then 2ml of concentrated sulphuric acid was added and heated for 2 minutes, a greyish coloration indicate the presence of steroid.

Test Terpenoids: Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated sulphuric acid was added and heated for about 2 minutes, a greyish color indicate the presence of terpenoids.

Test for Alkaloids

Mayer's Test: Crude extract was mixed with 2ml of 1% hydrochloric acid and heated gently. Mayer's reagent was added to the mixture, turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Wagner's Test: Crude extract was mixed with 2ml of 1% hydrochloric acid and heated gently. Wagner's reagent was added to the mixture, turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Quantitative Analysis of Formulation

Alcohol Content by Spectrophotometry (Dichromate Method)

Alcohol was estimated by dichromate method. For calibration, a standard curve was prepared using absolute alcohol without distillation (Table 4) & Figure 1.

Table 4 Organoleptic characteristics of Laportea Arishta

Parameter	Description
Colour	Brown
Odour	Alcoholic
Taste	Sweet, Astringent
Appearance	Clear

Preparation of Sample Solution: 1ml sample with 25 ml dichromate reagent was taken in a distillation flask 500ml (34g of potassium dichromate taken in distilled water in 1L Volumetric flask, then added 325ml of concentrated sulphuric acid drop wise to the flask). Then 25ml distillate was collected and incubated the flask (60° C for 29 min) and then measured the absorbance at 620nm in UV visible spectrophotometer[2,5].

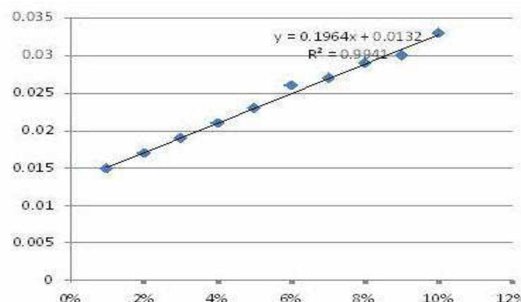

Figure 1 Standard Plot of Absolute Ethanol

Total Reducing Sugar

Estimation of total reducing sugar was carried out as follows. 50ml distilled water and 3ml HCL were added to 5ml of sample. Cooled and neutralized with sodium carbonate. Volume was adjusted to 500ml with distilled water and titrated against 5ml Fehling's A and 5ml Fehling's B, followed by mixing them and making final volume 500ml using methylene blue as indicator.

Total Phenolic Content: Total phenol content of samples was measured as Gallic acid equivalents.

Preparation of Standard Solution

Gallic acid was taken about 1gm and dissolved in 1000ml of distilled water, which is the stock solution (1mg/ml or 1000μg/ml) and from the stock solution 1ml, 2ml, 3ml, 4ml, 5ml (10,20,30,40,50 μg/ml) were taken to 100ml standard flask and made with distilled water.

5ml of folin ciocalteau reagent and 4ml of 1M sodium carbonate were added to 1ml of standard serial dilution, then incubated at dark for 30 minutes and then absorbance were measured at 765nm in UV visible spectrophotometer (ELICO SL 164 UV-Visible spectrophotometer) and a linear graph was plotted using the absorbance of standard serial dilutions and found out the phenol content[6]. The standard calibration plot was mentioned in Figure 2.

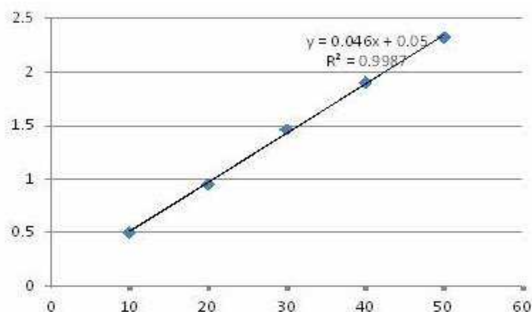

Figure 2 Standard Gallic Acid

Preparation of Sample Solution

5ml of folin ciocalteau reagent and 4ml of 1M sodium carbonate were added to 1ml of sample, then incubated at dark for 30 minutes and then absorbance were measured at 765nm in UV visible spectrophotometer (ELICO SL 164 UV-Visible spectrophotometer)

Total Flavanoid Content: Total Flavanoid content of samples was measured as Quercetin equivalents.

Preparation of Standard Solution

Quercetin was taken about 1gm and dissolved in 1000ml of methanol, which was the stock solution (1mg/ml or 1000µg/ml) and from the stock solution 1ml, 2ml, 3ml, 4ml, 5ml (100, 200, 300, 400, 500 µg/ml) were taken to 10ml standard flask and made up with methanol.

Standard serial dilution absorbances were measured at 420nm in UV visible spectrophotometer and linear graph was plotted using the absorbance of standard serial dilutions and found out the Flavanoid content.

Preparation of Sample Solution

Aluminum chloride colorimetric method was used for the determination of Flavanoid content. 1ml of sample was mixed with 3ml of methanol, 0.2ml of 10% aluminum chloride, 0.2ml of potassium acetate and 5.6ml of distilled water, then incubated at room temperature for 30 minutes and then absorbance were measured at 420nm in UV visible spectrophotometer .

Organoleptic and Physicochemical analysis of formulation:

Measurements of Physico-chemical properties can be useful in understanding the radical scavenging efficiency of herbal extracts (Table 5 & 6).

Table 5 Physico chemical Analysis of the formulation

Test	Observed Value
Total Ash	0.012(g)
Acid Insoluble Ash	0.029(g)
Water Insoluble Ash	0.009(g)
Sulphated Ash	0.031(g)
pH of Formulation	4.12 ± 0.26
Acid Value	0.022± 0.0009
Alcohol Content	2.2 ± 0.02 (%w/w)
Total Solid Content	0.3(g)
Viscosity of Formulation	1.136 (cps)
Refractive Index of Formulation	1.3532 ± 0.0001

Organoleptic character determination

Color, odor, taste and appearance of formulation checked out manually[2].

Table 6 Quantitative Phytochemical analysis of Formulation

Constituents	Ethyl extract formulation
Alcohol Content	5.6 %
Total Reducing Sugar	12.54%
Total phenolic content	26 ± 4(mg/g)
Total Flavanoid Content	240 ± 10 (mg/g)

Physicochemical analysis

Total Ash Value: Silica crucible heated and allows to cool and weighed. About 1 gm formulation was ignited to constant weight[4].

Acid Insoluble Ash: The total ash was boiled with 25ml of hydrochloric acid for 5min, collected the insoluble matter in ash less filter paper, washed with hot water, ignited, cooled and weighed.

Water Insoluble Ash: The total ash was boiled for 5 min with 25 ml water, collected the insoluble matter in ash less filter paper, washed with hot water ignited, cooled and weighed.

Sulphated Ash: 2gm of formulation was taken in silica crucible and 3 ml of sulphuric acid was added and incinerated until free from carbon.

pH : The digital pH meter was used and calibrated using buffer tablets of pH 4.0 and PH 7.0[6].

Acid Value: 10g of formulation was dissolved in 50ml of equal volume of ethanol and ether previously neutralized with 0.1M KOH to phenolphthalein solution. To it 1ml of phenolphthalein was added and titrated with 0.1M KOH[4,6].

Alcohol Content: 25ml of formulation was transferred to distillation flask and added equal volume of water into it. Afterwards it was distilled and distillate less than 2ml was collected and made up with distilled water. Specific gravity of this distillate was determined and alcohol content was analyzed using relative density.

Total Solid Content: 10 g of formulation was taken in previously weighed china dish and allowed to evaporate so that only solid content remained. Then it was again weighed to find out the solid content.

Viscosity of Formulation: Viscosity was determined with help of Ostwald viscometer. Viscometer was cleaned with distilled water, then dried and fixed on a stand firmly in vertical position. Transferred known amount of water through wide limb and sucked through other limb upto a level higher than the upper mark A and clamped the rubber tube with finger. The rubber tube was released and allowed water to flow down. The stop clock was started when water passes the mark A and stopped the stop clock when water passes the mark B. The time taken was noted and repeated with sample.

$\mu_1/ \mu_2 = t_1 \rho_1 /t_2 \rho_2$

Where, μ_1 and μ_2 are viscosity of sample and water

t_1 and t_2 are time of flow of sample and water

ρ_1 and ρ_2 are density of sample and water

Refractive Index: Refractive index of formulation was found out using Abbes' Refractometer.

Anti-oxidant Activity Determination of Formulation
Ferric Thiocyanate (FTC) Method
Preparation of Sample Solution

4ml of formulation were mixed with 4ml of absolute ethanol, 4.1ml of 2.52% linoleic acid in absolute ethanol, 8ml of 0.02M phosphate buffer (pH 7.0) and 3.9ml of distilled water and the mixture was placed at 40°c (0.1ml) and was then mixed with 9.7ml of 75% (v/v) ethanol and 0.1ml of ammonium thiocyanate and 3 minutes after added 0.1ml of 0.02M ferrous chloride, the absorbance was measured at 500 nm in spectrophotometer (ELICO SL 164 UV-Visible spectrophotometer) (Table: 7).

Table 7 Anti-oxidant Activity Determination of Formulation

Test	Result
FTC	74 ± 0.2%
TBA	80 ± 0.3%
Total Antioxidant activity by FRAP	3200 ± 50µg/ml

Preparation of Standard Solution

4mg of Vitamin C was mixed with 4ml of absolute ethanol, 4.1ml of 2.52% linoleic acid in absolute ethanol, 8ml of 0.02M phosphate buffer (pH 7.0) and 3.9ml of distilled water and the mixture was placed at 40°c (0.1ml) and was then mixed with 9.7ml of 75% (v/v) ethanol and 0.1ml of ammonium thiocyanate and 3 minutes after add3e 0.1ml of 0.02M ferrous chloride, the absorbance was measured at 500nm in spectrophotometer (ELICO SL 164 UV-Visible spectrophotometer)[2].

Equation for Determining Antioxidant Activity by FTC Method is given below:

$$\%\text{inhibition} = 100 - [(A_1 - A_0) \times 100]$$

Where, A0 is the absorbance of control
A1 is the absorbance of sample

Thiobarbituric Acid (TBA) Method
Preparation of Sample Solution

2ml formulation on the final day of the FTC assay was added to 1ml of 20% aqueous Thiobarbituric acid. After boiling for 10 minutes, the sample was cooled and absorbance was measured at 532nm in U.V -Visible spectrophotometer.

Preparation of Standard Solution

2ml standard on the final day of the FTC assay was added to 1ml of 20% aqueous Thiobarbituric acid. After boiling for 10 minutes, the sample was cooled and absorbance was measured at 532nm in spectrophotometer.

The equation for determining antioxidant activity by TBA Method was given below.

$$\%\text{inhibition} = 100 - [(A_1 - A_0) \times 100]$$

Where, A0 is the absorbance of control
A1 is the absorbance of sample

Total Antioxidant Activity By FRAP Method

Ferric reducing antioxidant potential (FRAP) assay was carried out using modified method of benzie and strain (1996). The result was expressed in Fe (II)/g dry mass.

Preparation of FRAP Reagent

FRAP reagent was prepared freshly by mixing 25ml acetate buffer pH 3.6, 2.5ml of TPTZ, 2.5ml of 20mM ferric chloride hexahydrate.

Preparation of Sample Solution

2850µl of FRAP reagent were heated to attain 37°c and then allowed to react 150 µl formulation for 30 minutes in dark. Then the absorbance was measured in 593nm (ELICO SL 164 UV-Visible spectrophotometer).

Preparation of Ferrous Sulphate Standard Plot

Ferrous sulphate was taken about 1gm and dissolved in 100ml of water, which was the stock solution (10mg/ml or 10000µg/ml) and from the stock solution 1ml, 2ml, 3ml, 4ml, 5ml (1000,2000,3000,4000,5000 µg/ml) were taken to 10ml standard flask and made with water. Standard serial dilution absorbances were measured at 593nm in UV visible spectrophotometer and a linear graph was plotted using the absorbance of standard serial dilutions and found out the ferrous sulphate content.

Validation of formulation by UV

A new, simple, sensitive, selective, and precise UV method for analysis of Eriocitrin and Quercetin in formulation was developed and validated

Specification of UV

Model	ELICO SL 164
Instrument	UV-Visible Double Beam Spectrophotometer
Wavelength Range	190-999.9 nm
Accuracy	±0.5nm
Repeatability	±0.2nm
Resolution	0.1nm
Bandwidth	0.5-6.0 nm (variable at an interval of 0.1nm)
Photometric range	-2.5 to +2.5 Abs
Accuracy	±0.005 abs at 0.1abs from 200-850nm
Repeatability	±0.002 abs at 1.0 abs from 200-850nm
Stray light	less than 0.05% at 220-340nm
Source	Tungsten- Halogen lamp (310-999.9nm)
Detectors	Photomultiplier Tube (PMT)

The *Laportea arishta* was validated by UV-visible spectrophotometer with respect to linearity, precision, accuracy, robustness, ruggedness, limit of detection, limit of quantification by using standard Eriocitrin and Quercetin[4]. The UV spectrums and method validation summary of Eriocitrin and Quercetin were mentioned from Table 8 to Table 11.

Linearity

Working dilutions of standard Eriocitrin in the range of 1-5 µg/ml and Quercetin in the range of 1-5 µg/ml were prepared by taking suitable aliquots of working standard solutions in different 10ml of volumetric flasks and diluted up to the mark with water and methanol respectively and then standard Eriocitrin was measured at 221nm and

standard Quercetin was measured at 310nm for obtaining absorbance's of various concentrations. The experiment was performed six times and the mean was used for the calculations (Table: 12).

Table 8 From UV Spectrum No 1

Sr. No	Wavelength (nm)	Absorbances
1	221	1.812
2	242	2.663
3	263	2.770
4	291	2.861
5	310	2.823
6	354	2.837
7	368	2.800
8	424	1.628

Table 9 From UV Spectrum No 2

Wavelength (nm)	Absorbances
221	1.714

Table 10 Method Validation Summary of Eriocitrin

Parameters	Standard
Linearity	1-5 (µg/ml)
Slope	0.146
Intercept	0.127
Correlation Coefficient	0.999
LOD (µg/ ml)	0.904
LOQ (µg/ ml)	2.739

Table 11 Method Validation Summary of Quercetin

Parameters	Standard
Linearity	1-5 µg/ml
Slope	0.188
Intercept	0.0127
Correlation Coefficient	0.99
LOD µg/ ml	0.98
LOQ µg/ ml	2.9

Precision: Precision of analytical method was studied by taking absorbances of homogenous sample (Table: 13).

Table 13 Precision Validation of Formulation by UV

	Eriocitrin (µg/ml)	%RSD	Quercetin (µg/ml)	%RSD
Intra-Day Precision	5	0.065	5	0.0577
Inter-Day Precision	5	0.091	5	0.041

Intra-day precision: Absorbance's of same concentration was taken six times at same day.

Inter-day precision: Absorbance's of same concentration was taken six times at three consecutive days.

Accuracy: Accuracy of the method was studied using the method of standard addition. Standard Eriocitrin and Quercetin solutions were added to the formulation. The percent recovery was determined at three different levels 80% and 100% and 120%. Eriocitrin and Quercetin content were determined and the percent recovery was calculated (Table: 14).

Robustness & Ruggedness: Robustness was studied by changing parameters like temperature and Ruggedness was done by taking absorbances of standard Eriocitrin and Quercetin by two analysts(Table: 15).

Table 14 Accuracy Validation of Formulation by UV

Standard	Std % (%)	Mean absorbance	%Recovery (%)
Eriocitrin	80	2.059	98.71
Eriocitrin	100	2.164	99.93
Eriocitrin	120	2.295	100.95
Quercetin	80	1.101	99.65
Quercetin	100	1.139	99.0
Quercetin	120	1.154	99.3

Limit of detection (LOD) & Limit of quantification (LOQ): Limit of detection (LOD) is the lowest amount of analyte in the sample that can be detected and Limit of quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined by suitable precision and accuracy (Table: 16).

Results and Discussion

Physicochemical analysis of the crude drug of *Laportea interrupta (L) Chew leaves* showed no rodent contamination, no foreign organic matter, no insect infestation. Total ash value, Acid insoluble ash, Water insoluble ash, Sulphated ash and moisture content were under the limit.

Laportea interrupta (L) Chew leaves were extracted in Soxhlet apparatus using ethyl acetate, petroleum ether, chloroform, and methanol and aqueous. The ethyl acetate extract found more yield when compared to other extracts. So that extract was incorporated in the preparation of Arishta. Different phytochemical analysis were performed for the formulation and it was found positive results for carbohydrates, phenols and tannins, flavanoids, glycosides, steroids in qualitative phytochemical analysis . Evaluation of preliminary organoleptic characteristics revealed that the prepared arishta is of brown color, alcoholic odour, sweet followed by an astringent taste .pH and acid value indicated that the formulation have weak acidic properties. The basic tools for the quality control measurement of arishtas are alcohol percentage, solid content, viscosity, refractive index and specific gravity. The acceptance of arishtas mainly dependent on the above contents

Anti-Oxidant activity of the arishta showed 74 ± 0.2% by ferric thiocyanate (FTC) method, 80 ± 0.3% by Thiobarbituric acid (TBA) method and total antioxidant activity by FRAP method gave 3200 ± 50 µg/ml equivalent to standard ferrous sulphate. Here a sensitive, precise and accurate UV method validation of the formulation was performed by using standard Eriocitrin and Quercetin. The LOD of Eriocitrin and Quercetin were 0.904 and 0.98 µg/ml and LOQ of both were 2.739 and 2.9 µg/ml respectively.

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Table 12 Linearity Validation of Formulation by UV

Sl No	Eriocitrin (µg/ml)	Absorbance	%RSD	Quercetin (µg/ml)	Absorbance	%RSD
1	1	0.027	1.48	1	0.2	0.281
2	2	0.167	0.239	2	0.397	0.141
3	3	0.319	0.125	3	0.581	0.096
4	4	0.465	0.086	4	0.740	0.075
5	5	0.61	0.065	5	0.97	0.0577

Table 15 Robustness and Ruggedness

Parameter	Conc (µg/ml) Eriocitrin	Abs	%RSD	Conc (µg/ml) Quercetin	Abs	%RSD
Room temp	5	0.91	0.061	5	0.97	0.057
Cold temp	5	0.61	0.065	5	0.97	0.041
Analyst 1	5	0.61	0.065	5	0.97	0.057
Analyst 2	5	0.61	0.049	5	1.038	0.061

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