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VALIDATION OF NEWLY FORMULATED LAPORTEA ARISHTA BY USING DIFFERENT ANALYTICAL METHODS

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ABSTRACT

Traditional medicines have nurtured the knowledge of natural remedies against diseases since ages. Growing awareness about harmful side effects of modern medicine has led to interest in ayurveda. Laportea interrupta (L) Chew of family Urticaceae having common name Hawaii woodnettle is an herb having many traditional uses. As a preliminary step physicochemical analysis of crude drug, Laportea interrupta-leaves were performed. Physicochemical analysis includes Rodent Contamination, Foreign Organic Matter, Insect Infestation Total Ash Value, Acid Insoluble Ash, Water Insoluble Ash, Sulphated Ash and Moisture Content. Laportea interrupta leaves extracted in soxhlet apparatus using ethyl acetate, petroleum ether, chloroform, methanol and water for identification of constituents by qualitative phytochemical analysis (tests for protein, carbohydrates, phenols and tannins, flavanoids, saponins, glycosides, steroids, terpenoids and alkaloids) and quantitative phytochemical analysis (total phenol content and total flavanoid content). The collected leaves of Laportea interrupta was used to prepare arishta. After preparing the arishta the organoleptic characteristics (colour, odour, taste and appearance), the physicochemical analysis (p^H, acid value, alcohol content, total solid content, viscosity and refractive index), quantitative analysis (alcohol content by spectrophotometry by dichromate method, total reducing sugar, total phenol content and total flavanoid content), anti-oxidant activity determination (Ferric Thiocyanate method, Thiobarbituric acid method and Total Antioxidant Activity by FRAP method) were performed. LCMS was performed for determining various constituents (Qualitative analysis) and standardisation of formulation was done by using UV and HPLC. The above formulated *Laportea Arishta* was validated by using different analytical equipment'sas per ICH guidelines.

Keywords: Arishta, Physicochemical analysis, Organoleptic characteristics

INTRODUCTION

Herbal medicine (or "Herbalism") is the study and use of medicinal properties of plants. They have the ability to synthesize a wide variety of chemical compounds that are used to Herbal medicine (or "Herbalism") is the perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases. Herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects.

Ayurveda - The Natural Way of Treating Disease Ayurveda is a form of alternative medicine that uses different parts of herbs, plants and parts of animals to cure diseases and ailments. The word Ayurveda comes from two Sanskrit words, 'ayus' meaning life and 'Veda' meaning knowledge. Ayurveda is a complete and highly integrated science of life. It is a holistic and complex spiritual and philosophical system which has been developed over thousands of years and is a subtle body of knowledge. This medical system is based on the system of prevention which means that people are often treated before the symptoms of the illness become evident.

Arishta and Asava have been used as medicines for over 3000 years to treat various disorders and are also taken as appetizers and stimulants. Due to their medicinal value, sweet taste, and easy availability people are prone to consume higher doses of these drugs for longer periods. Arishta are medicinal preparations made by soaking the drugs in coarse powder form or in the form of decoction (Kashaya), in a solution of sugar or Validation is documenting that a process meets its pre-determined specifications and quality attributes the objective of validation of an analytical procedure is to demonstrate that it is

jaggery, as the case may be, for a specified period of time, during which it undergoes a process of fermentation generating alcohol, thus facilitating the extraction of the active principles contained in the drugs.

Laportea interrupta (L) Chew of family Urticaceae having common name Hawaii woodnettle is an herb having many traditional uses like Allergies, Alopecia, Amenorrhea, Arthritis, Asthma, Bedwetting/incontinence, Female Hormones, Fibromyalgia, Libido, Longevity/tonics, Menorrhagia, Nutrition, Osteoporosis, PMS, Prostate, Rheumatoid arthritis.

Flavanoids are natural products widely distributed in plant kingdom and currently consumed in large amounts in the daily diet. Flavanoids have been reported to exert wide range of biological activities like anti-inflammatory, antibacterial, antiviral, anti-allergic, cytotoxic antitumor, treatment of neurodegenerative diseases, vasodilatory action. In addition they are known to inhibit lipid-peroxidation, platelet aggregation, capillary permeability and fragility, cyclo-oxygenase and lipoxygenase enzyme activities. They exert these effects as antioxidants, free radical scavengers, chelators of divalent cation. These are also reported to inhibit variety of enzymes like hydrolases, hyalouronidase, alkaline phosphatise, arylsulphatase, cAMP phosphodiesterase, lipase, α-glucosidase, kinase.

Standardization is the development and implementation of concepts, doctrines, procedures and designs to achieve and maintain the required levels of compatibility, interchangeability or commonali ty in the operational, procedural, material, technical and administrative fields to attain interoperability. These standardization processes create compatibility, similarity, measurement and symbol standards.

suitable for its intended purpose. A tabular summation of the characteristics applicable to identification, control of impurities and assay procedures is included. Other analytical procedures may be considered in future additions to this document. Typical validation characteristics which should be considered are accuracy, precision, repeatability, intermediate precision, specificity, detection limit, quantisation limit, linearity, range.

MATERIALS AND METHOD

Collection of plant material

The leaves of Laportea interrupta were collected 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

Total ash value: About 1 gm dried powder ignited to constant weighed.

Acid insoluble ash: Boiled the total ash with 25ml of hydrochloric acid ignited, cooled and weighed.

Water insoluble ash: Boiled the total ash for 5 min with 25 ml water, ignited, cooled and weighed.

Sulphated ash: 2gm of powdered drug was taken in 3 ml of sulphuric acid incinerated until free from carbon.

Moisture content: 10 gm. sample was dried in oven at 100° C, cooled and weighed .

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. (Harborne, J.B., 2007)

Preparation of the Extracts: The coarse leaf powder of Laportea interrupta was taken about 20gm and extracted with 250ml of ethyl acetate (70-80°c), 250ml of Petroleum Ether (60-80°c), 250ml of Chloroform (50.5-61.5°c), 250ml of methanol (60-70°c) and 250ml of Distilled water (0.25%) by continuous hot percolation using soxhlet apparatus.

Qualitative phytochemical analysis

The extracts were subjected to qualitative tests for identification of phytochemical constituents present in it. (RNS Yadev et al. 2011)

Test for protein

Million'sTest: Crude extract was mixed with 2ml millions reagent and heated.

Ninhydrin Test: Crude extract was boiled with 2ml of 0.2% solution of ninhydrin.

Test for carbohydrates

Fehling's Test: Equal volume of Fehling's A and B reagent mixed together and 2ml of it added to crude extract and heated gently.

Benedict's test: Crude extract mixed with 2ml Benedict reagent and boiled.

Molisch's Test: Crude extract was mixed with 2ml molisch reagent and added 2ml concentrated sulphuric acid along the sides of test tube.

Iodine Test: Crude extract was mixed with 2ml iodine solution.

Test for phenols and tannins

Ferric Chloride Test: Crude extract was mixed with 2ml of 2% solution of ferric chloride.

Lead Acetate Test: Crude extract was mixed with 2ml of 10% lead acetate solution.

Test for flavanoids

Shinoda Test: Crude extract was mixed with few fragments of magnesium ribbon and concentrated hydrochloric acid was added drop wise.

Alkaline Reagent Test: Crude extract was mixed with 2ml of 2% solution of sodium hydroxide.

Test for saponins: Crude extract was mixed with 5ml distilled water in a test tube and shaken vigorously.

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.

Salkowski's Test: Crude extract was mixed with 2ml of chloroform and then 2ml of concentrated sulphuric acid was added carefully and gently shaken.

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.

Test for steroids: Crude extract was mixed with 2ml of chloroform and concentrated sulphuric acid was added sidewise. Mixing crude extract with 2ml chloroform, then 2ml of concentrated sulphuric acid was added and heated for 2 minutes.

Quantitative phytochemical analysis

Total Phenol Content: The amount of phenol in the ethyl acetate extract was determined by Folin-ciocalteu reagent method with some modification.5ml of 10% Folin-ciocalteau reagent and 4ml of 1M sodium carbonate added to 0.1ml extract and to 1ml of standard serial dilution of standard Gallic acid, then incubated at dark for 30 minutes and then absorbance were measured at 765nm.

Total Flavanoid Content: The amount of phenol in the ethyl acetate extract was determined by Aluminium chloride colorimetric method with some modification. 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 absorbance were measured at 420nm.

PREPARATION OF FORMULATION

The earthen pot sufficiently large and strong is chosen. The proportion of the different ingredients are 32 seers of distilled water, boiled and added 1.25 seers dried plant leaf powder to make decoction,to this decoction added jaggery 12.5 seers, honey 6.25 seers and sugar 6.25 seers, mixed well to form a uniform solution and then poured to the prepared earthen pot mixture upto its three by forth, then lid is closed and sealing is done by cleaned ribbon. Pot is buried in a pit made in the soil for 30 days, filtered through a muslin cloth and stored in tightly stoppered glass bottles.

Test for 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.

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.

Wagner's Test: Crude extract was mixed with 2ml of 1% hydrochloric acid and heated gently. Wagner's reagent was added to the mixture.

Physicocemical analysis of formulation

Determination of P^H of formulation: The digital P^H meter was used and calibrated using buffer tablets of P^H 4.0 and P^H7.0.

Determination of 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 added and titrated with 0.1M KOH.

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

Total solid content: 10 g of formulation was allowed to evaporate so that only solid content remained. Then it weighed to find out the solid content

Determination of viscosity of formulation: Viscosity determined with help of Ostwald viscometer.

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

QUANTITATIVE ANALYSIS OF FORMULATION

Alcohol content by spectrophotometry (Dichromate Method): Alcohol was estimated by caputi et al (1968). For calibration a standard curve prepared using absolute alcohol without distillation.1ml sample with 25 ml dichromate reagent taken in a distillation flask and distilled to obtain about 25ml distillate, Incubated the flask at 60° C for 29 min and Measured the absorbance at 620nm.

Total reducing sugar: 50ml distilled water and 3ml HCL was added to 5ml of sample then cooled and neutralized with sodium carbonate and 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 a indicator.

Total phenol content: The amount of phenol in the formulation was determined by Folin-ciocalteu reagent method with some modification. 5ml of 10% Folin-ciocalteau reagent and 4ml of 1M sodium carbonate added to 1ml extract and to 1ml of standard serial dilution of standard Gallic acid, then incubated at dark for 30 minutes and then absorbance were measured at 765nm and the results were determined from the standard curve and expressed in gallic acid equivalents (mg/g of extracted compound)..

Total flavanoid content: The amount of phenol in the formulation was determined by Aluminium chloride colorimetric method with some modification. 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 absorbance were measured at 420nm and the results were determined from the standard curve and expressed in Quercetin equivalents (mg/g of extracted compound).

ANTI-OXIDANT ACTIVITY DETERMINATION OF FORMULATION

Ferric Thiocyanate (FTC) method: 4ml of formulation and 4mg of Vitamin C 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, 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, after 3 minutes 0.1ml of 0.02M ferrous chloride were added and the absorbance was measured at 500nm.

Thiobarbituric Acid (TBA) method: 2ml formulation and 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.

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

Qualitative analysis of formulation by lcms

Specifications of LCMS are LC Column: REVERSE PHASE C-18, Pump: SPD 10 AVP, Mobile Phase: 2% OPA IN WATER: METHANOL (50:50), Ionisation Mode: ELECTRONIC SPRAY IONIZATION, Mode: BOTH POSITIVE, Injection Volume: 10 µL, Flow Rate: 2 ML/MIN, Column Temperature: 250°C, Column: PHENOMENEX RP 18, Column Dimension: 25CM × 2.5 MM,LC Detection: 254NM,m/z range: 50-1000,Soft Ware: CLASS V P INTEGRATED AND Library: METWIN 2.0.

Standardisation of formulation using UV

Specifications of UV are 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,Base line correction: Automatic Base Line Correction, Scan speed: Slow, Medium and Fast, Data interval: Depend Upon Wavelength, Sample holder: 5 Position, Specification of HPLC is Instrument: Schimadzu, LC Column: Reverse Phase C-18 Phenomenex C18, Pump: SPD 10 AVP, Mobilephase: Acetonitrile: Methanol (40:60), Injection volume: 10 µl, Flow Rate: 2.0 ml/min, Column Temperature: 25°C, Column: Automatic positioning far 10mm and Sample Cuvette and Fixed position for Reference Cuvette, Source: Tungsten- Halogen lamp (310-999.9nm) and Deuterium lamb with Quartz window (190-340nm) and Detectors: Photomultiplier Tube (PMT).Validation experiments were performed to demonstrate linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ as per ICH guidelines.

Standardisation of formulation using HPLC

Phenomenex RP 18, Column Dimension: 5cm × 1.5 cm,LC detection: 254nm and Soft Ware: Class V P Integrated.Validation experiments were performed to demonstrate system suitability, linearity, precision, accuracy study and robustness as per ICH guidelines

RESULT AND DISCUSSION

Table 1: Physicochemical analysis of crude drug

TEST	RESULT	INFERENCE
Rodent Contamination	Absent	Should be Absent
Foreign Organic Matter	0.54%	Not more than 2%
Insect Infestation	Absent	Should be Absent
Total Ash	0.16g	Present in limit
Acid Insoluble Ash	0.33g	Present in limit
Water Insoluble Ash	0.11g	Present in limit
Sulphated Ash	0.36g	Present in limit
Moisture Content	0.235g	Present in limit

Table 2: Quantitative phytochemical analysis

S. No	Constituents	Ethyl acetate extract (mg/g)
1	Total phenol	38+/- 4
2	Total flavanoid	260+/-10

Table 3: Qualitative phytochemical analysis

S No	Constituents	Ethyl - acetate	Pet. Ether	chloroform	methanol	water
1	Proteins	-VE	-VE	-VE	-VE	-VE
2	Carbohydrates	+VE	-VE	-VE	+VE	+VE
3	Phenols and Tannins	+VE	-VE	-VE	+VE	+VE
4	Flavanoids	+VE	-VE	-VE	+VE	-VE
5	Saponins	-VE	-VE	-VE	-VE	-VE
6	Glycosides	+VE	-VE	-VE	+VE	+VE

7	Steroids	+VE	+VE	-VE	+VE	-VE
8	Terpenoids	-VE	+VE	+VE	-VE	-VE
9	Alkaloids	+VE	-VE	-VE	+VE	-VE

STANDARDISATION OF FORMULATION

Table 4: Organoleptic characteristics of formulation

S NO	PARAMETER	DESCRIPTION
1	Color	Brown
2	Odour	Alcoholic
3	Taste	Sweet, Astringent
4	Appearance	Clear

Table 5: Physicochemical analysis of formulation

TEST	OBSERVED VALUE
Total Ash	0.012g
Acid Insoluble Ash	0.029g
Water Insoluble Ash	0.009g
Sulphated Ash	0.031g
P^H of Formulation	4.36 +/_ 0.26
Acid Value	0.022 +/_ 0.0009
Alcohol Content	2.2 +/_ 0.02 (%w/w)
Total Solid Content	0.3g
Refractive Index of Formulation	1.3532 +/_ 0.0001

Table 6: Qualitative analysis of formulation by LCMS

SL No	COMPOUND NAME	Mol mass	ACTIVITY
1	VALERIC ACID	102.14	Antioxidant, Anti-Inflammatory
2	10-HYDROXY-2-DECENOIC ACID	186.25	Anti-Tumor, Antidepressant
3	CHRYSOPHANIC ACID 9-ANTHRONE	240.26	Antifungal, Antimicrobial
4	DEOXYQUERCETIN	286.25	Antioxidant, Anti-Inflammatory, Anti-diabetic
5	QUERCETIN	302.24	Antitumor
6	QUERCETINDIHYDRATE	338.27	Smooth Relaxant, Diuretic, Anti-Inflammatory
7	1,9-DIDEOXYFORSKOLIN	378.51	Antihypertensive, Positive Inotropic.
8	FRIEDELIN	426.73	Anti-Inflammatory, Analgesic, Antipyretic.
9	CAROTENE-EPOXIDE	552.89	Anticancer
10	ERIOCITRIN	596.54	Potent Antioxidant
11	METHYL AMINO L ALANINE	118.14	Nuerotoxic
12	DECCANOIC ACID	172.27	
13	ENICOFLAVINE	211.22	Hypoglycaemic, Hypolipidemic
14	DEOXY KAEMPFEROL	270.25	Antioxidant, anti-inflammatory, anti-tumour, antidepressant, antimicrobial, anti- diabetic, cardio protective, nueroprotective, anxiolytic, analgesic, antiallergic activity.

15	KAEMPFEROL	286.24	Antioxidant, anti-inflammatory, anti-tumour, antidepressant, antimicrobial, antidiabetic, cardio protective, nueroprotective, anxiolytic, analgesic, antiallergic activity.
16	HERBACETIN	302.27	Anti-Inflammatory
17	STIGMASTEROL	412.70	Antitrypanosomal, Anti-Hypercholesteroane
18	KAEMPFEROL 3-GLUCOSIDE	448.38	Reduces Body Weight
19	PALMITOYL ACETATE	660.90	Anti-Inflammatory

Table 7: Quantitative analysis of formulation

TEST	OBSERVED RESULT
Alcohol Content	5.6 %
Total Reducing Sugar	12.54%
Total phenol content	26 +/_ 4mg/g
Total Flavanoid Content	240 +/_ 10mg/g

Table 8: Anti-oxidant activity determination of formulation

S. NO	TEST	RESULT
1	FTC	74 +/- 0.2%
2	TBA	80 +/_ 0.3%
3	Total Antioxidant activity by FRAP	3200 +/_ 50µg/ml

VALIDATION OF FORMULATION USING HPLC

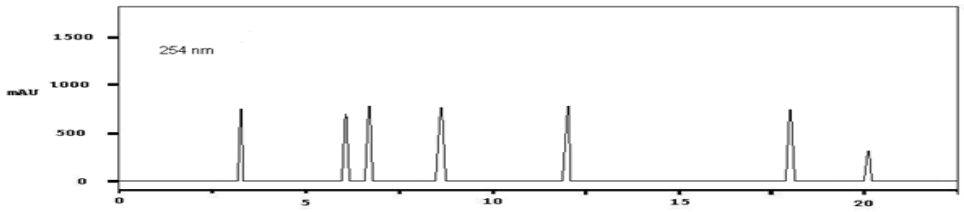

Fig:1 HPLC chromatogram of formulation

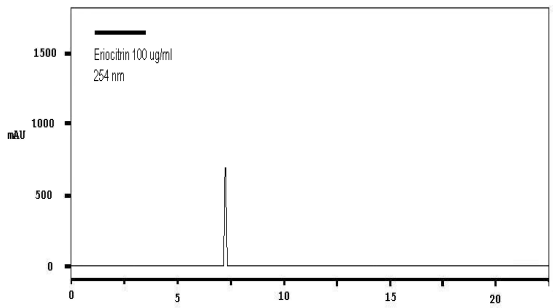

Fig:2 HPLC chromatogram of standard eriocitrin

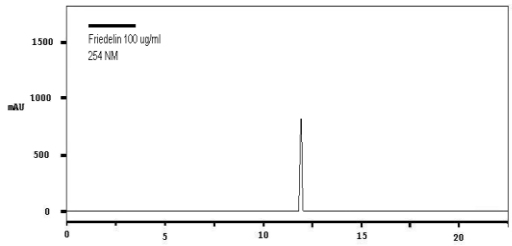

Fig: 3 HPLC chromatogram of standard friedelin

Table 9: Method validation summary

PARAMETERS	STANDARD
LINEARITY	40-120µg/ml
SLOPE	2.055
CORRELATION COEFFICIENT	0.994
LOD µg/ ml	0.08
LOQ µg/ ml	0.27

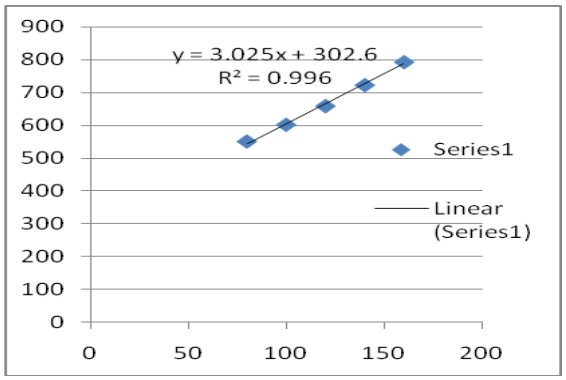

Fig:4 Linearity Plot Of Eriocitrin

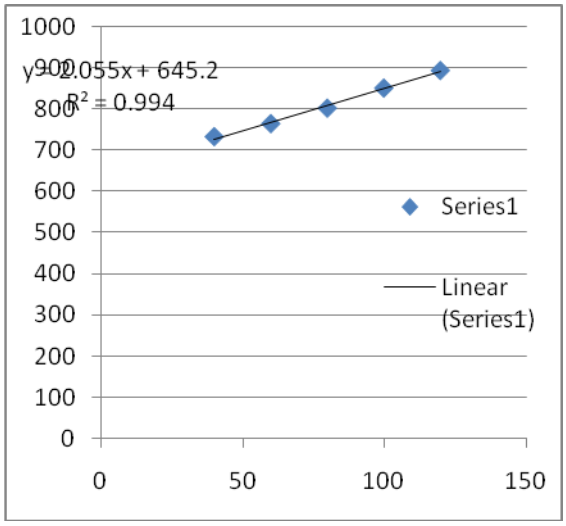

Fig:5 Linearity Plot Of Friedelin

Table 10: Accuracy

Standard	Std %	AUC	%RSD
Eriocitrin	80%	676	0.03
Eriocitrin	100%	702	0.032
Eriocitrin	120%	730	0.033
Friedelin	80%	872	0.30
Friedelin	100%	896	0.29
Friedelin	120%	918	0.28

Table 11: Robustness & Ruggedness

Para Meter	CONC (µg/ml) Eriocitrin	AUC	RETEN TIME	%RSD	CONC (µg/ml) Friedelin	AUC	RETEN TIME	%RSD
Flow rate 2.0 ml/min	100	602	7.25	0.32	100	852	12.01	0.29
Flow rate 2.1 ml/min	100	662	7.21	0.28	100	937	12.01	0.03
Analyst 1	100	602	7.25	0.32	100	852	12.01	0.29
Analyst 2	100	602	7.25	0.32	100	852	12.01	0.29

Table 12: Limit of detection (lod) &limit of quantification (LOQ)

	Eriocitrin (µg/ml)	Friedelin (µg/ml)
Limit Of Detection (LOD)	0.061	0.08
Limit Of Quantification (LOQ)	0.185	0.27

VALIDATION OF FORMULATION USING UV

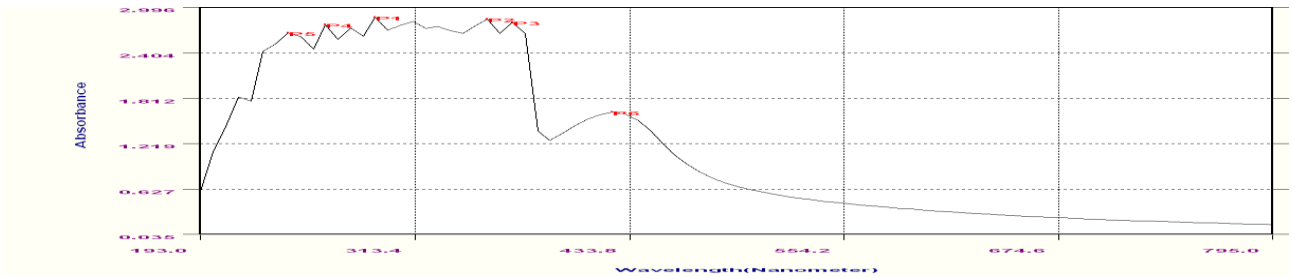

Fig:6 Chromatogram of Formulation

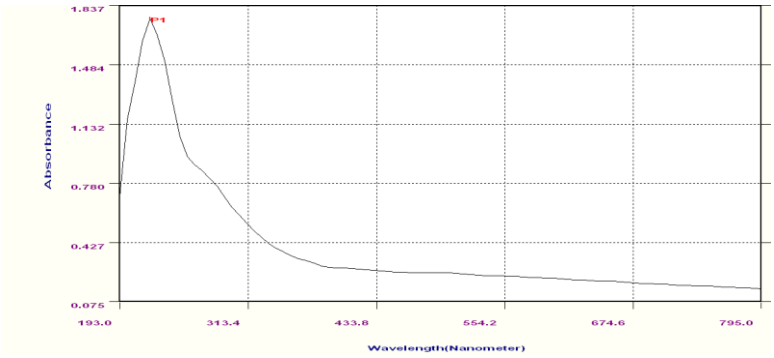

Fig:7 UV Spectrum of Standard Eriocitrin

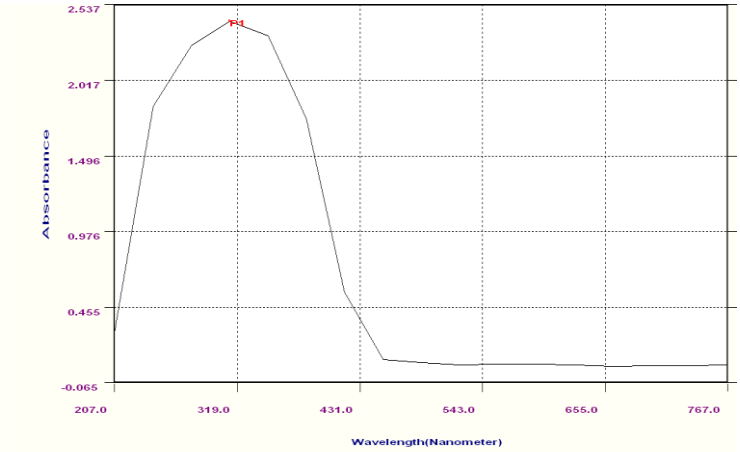

Fig:8 UV Spectrum of Standard Quercetin

Table 13: METHOD VALIDATION SUMMARY

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

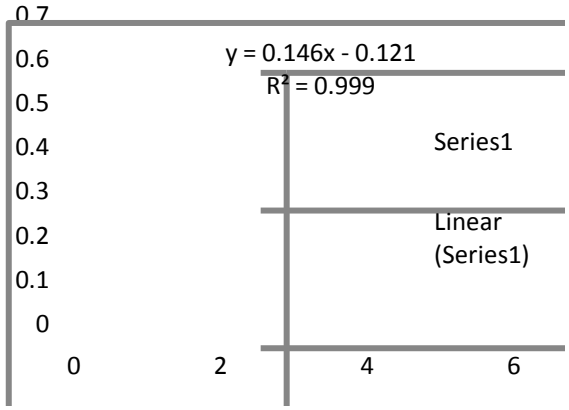

Fig: 9 Standard Plot Of Eriocitrin

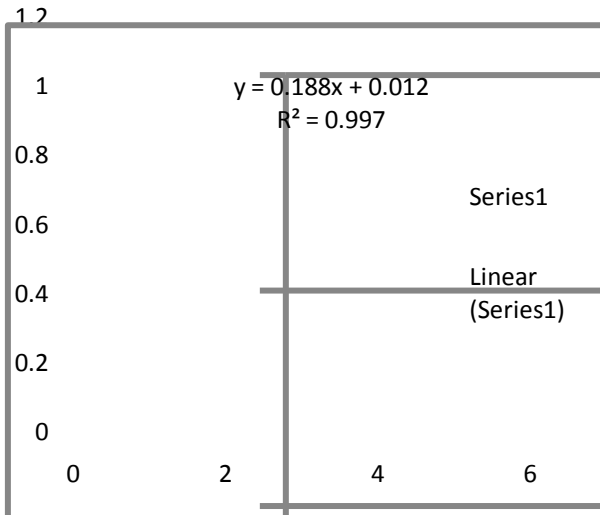

Fig: 10 Standard Plot Of Quercetin

Table 14: Precision

	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

Table 15: Accuracy

Standard	Std %	Mean abs	%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

Table 16: Robustness & ruggedness

Para Meter	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

Table 17: Limit of detection (LOQ) & Limit of quantification (LOQ)

	Eriocitrin (µg/ml)	Quercetin (µg/ml)
Limit Of Detection (LOD)	0.904	0.98
Limit Of Quantification (LOQ)	2.739	2.9

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