PHYTOCHEMICAL PROFILING OF THE AYURVEDIC MEDICINAL PLANT *Picrorhiza kurroa* Benth

KALEESWARI S

16PCH007

Thesis Submitted to

Avinashilingam Institute for Home Science and Higher Education for Women,

Coimbatore-641 043

In Partial Fulfilment of the Requirements for the Degree of

Master of Science in Chemistry

April 2018

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ACKNOWLEDGEMENT

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It is with the choice of blessings and the divine grace of **Lord Almighty** that any human endeavor is achieved.

I record my sincere thanks to **Padmashri, Dr.P.R.Krishnakumar,** Chancellor of Avinashilingam Institute for Home Science and Higher Education for Women, for provision of a conducive and congenial ambience for the execution of this research work

My grateful thanks to **Dr.Premavathy Vijayan,** Vice-Chancellor of Avinashilingam Institute for Home Science and Higher Education for Women, for the kind encouragement

My deepest thanks to **Dr.(Mrs) S.Kowsalya,** Registrar of Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for providing academic related facilities to carry out my work.

My sincere thanks to **Dr.(Mrs), A.Venmathi,** Former Registrar of Avinashilingam Institute for Home Science and Higher Education for Women, for providing permission to carry out my research work. .

My sincere thanks to **Dr. (Mrs) A.Parvathi,** , Dean, Faculty of Science, Avinashilingam Institute for Home Science and Higher Education for Women, for her valuable support and encouragement.

I express my special gratitude to **Dr. (Mrs.) R.Rajalaskshmi,** Professor and HOD Department of Chemistry, Avinashilingam Institute for Home Science and Higher Education for Women, for her encouragement and providing all lab facilities at any time throughout my study.

I specially acknowledge my deep sense of gratitude and respect to my guide **Dr.Shubashini K sripathi** , Professor, Avinashilingam Institute of Home Science and Higher Education for Women, for her meticulous care ,eminent guidance, enormous help, continuous encouragement throughout my project. I am thankful to her for her remarkable and inspirational thoughts at every stage of my work. I am grateful for her constant support for the successful completion of thesis work efficiently and effectively

I also wish to thank all the staff members of the Department of chemistry, Avinashilingam Institute for Home Science and Higher Education for Women, for their help and encouragement and I also thank lab assistants, Department of Chemistry for their help.

I acknowledge with thanks the services rendered by the Instrumentation facility at Madurai Kamaraj University, Vellore Institute of Technology and the ARL lab of Avinashilingam Institute for Home Science and Higher Educatio for Women.

On a personal note, my special tribute to my beloved parents, my sister, relatives for their encouragement during my entire study.

My special thanks to my friends who have been with me in all my stages of work and supported me to do my work successfully.

I wish to extend my heartfelt thanks to my well-wishers for their constant encouragement, support and help in completion of this project work successfully.

KALEESWARI S

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LIST OF ABBREVIATIONS

WHO	World Health Organization
TLC	Thin Layer Chromatography
UV-VIS	Ultra Violet and Visible Spectroscopy
1H NMR	Proton Nuclear Magnetic Resonance
13C NMR	Carbon Nuclear Magnetic Resonance
HPLC	High Pressure Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
IR	Infra Red
GC-MS	Gas Chromatography and Mass Spectrometry
Rt	Retention time
H_2SO_4	Sulphuric acid

INTRODUCTION

1. INTRODUCTION

Ayurvedic medicine probably predates any other healing tradition in existence today- even Chinese medicine. Seeds from plants indigenous to India have been found in the tombs of the Egyptian pharaohs. Travellers had carried information about Indias' plants through tier into chine, and Arabs had traded for Indian herbs before the birth of Islam. *Picrorhiza kurroa* commonly known as kutki belonging to the family *Schophulariaceace*, is a herb native to the Himalayan region, Pakistan and Nepal. The roots and rhizomes of this plant are medicinally the most important part and are in great demand in the various traditional and folk system of medicine .The powder, decoction, infusion, confection, and alcoholic extract of the roots and rhizomes are useful in treating various liver ailments: their anticholestatic effects are useful in treating dyspepsia, chronic diarrhoea, and infection of the upper respiratory tract and their antioxidant and immunomodulatory effect in treating fever. (Benerjee et al., 2008)

Phytochemical investigation of plants is the initial and crucial step in preparing ayurvedic medicines. According to their functions in plant metabolism, phytochemicals are basically divided into two groups,

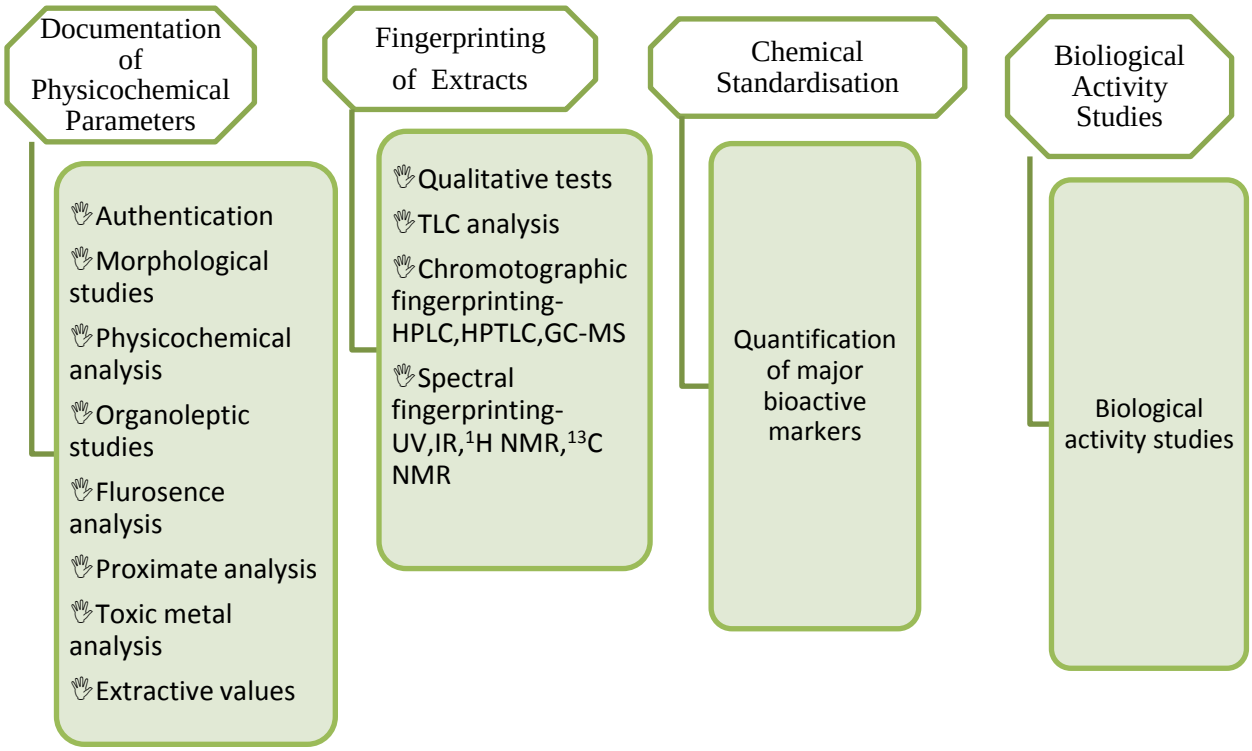
i) **Primary constituents:** comprises of common sugars, amino acid, proteins and chlorophyll. These constituents are essential for the life and growth of plants and herbs

ii) **Secondary constituents:** comprises of alkaloids, terpenoids, saponins, phenolic compounds, flavonoids and tannins.

The presence of the secondary metabolites in plants mainly accounts for the various use of plants in traditional medicine. Phytochemical screening involves qualitative colour tests for the various plant extracts. The class of natural product can be identified by colour tests (Harborne *et al.,*1973).The qualitative and quantitative estimation of the phytochemical constituents of a medicinal plant is considered to be an important step in the standardisation of medicinal plant extracts and herbal formulation. Spectral fingerprinting of extracts of medicinal plants is yet another standardisation strategy.

Standardisation of Medicinal Plants

Standardisation of medicinal plants involves the stages depicted in chart below,



Medicinal Plant Chosen for the Study: *Picrorhiza kurroa* Benth

1.2 Description of the Plant

Picrorhiza kurroa is an ayurvedic medicinal plant. It belongs to the plant family of ***Scrophulariaceae.***

Synonyms

- *Picrorhiza kurroa*

Common name

- Kardi,karoi,karu,karwi (Hindi)
- Katukhurohani (Malayalam)
- Kurk i(Nepali)
- Anjani,arishta,katumbhara (Sanskrit)
- Acokarokini,akutam,akutarokini,amakkini (Tamil)
- Katuka-rogani,katukarogani, katukkrohini (Telugu)
- Kutki (Urdu)

Distribution

- The species occurs in alpine Himalayas, from Kashmir to Sikkim.

Plant Description(www.herbal-supplemental-resource.com)

- The bitter tasting roots or pacrorhiza kurroa are hard, about 6-10inches in lengh, and creeping.
- The leaves are 2-4 inches long, oval in shape with a sharp apex, flat, and serrate.
- The flowers are whit or pale purple on a long spike, blooming in june through august.
- The fruit is a ½ inch long and oval in shape.
- The rhizome of picrorhiza is manually harvested in October through December

Plant Part Used:

Leaf, bark, root and rhizomes.

Figure (1) **Picture of the Chosen Plant (*Picrorhiza kurroa*)**

Objectives of study

- ✓ To carry out the phytochemical analysis of extracts of *Picrorhiza kurroa* Benth
- ✓ To determine the total phytoconstitutents (alkaloid, flavonoid,terpenoid and steroid)
- ✓ To document the chemical and spectral fingerprinting of extracts of *Picrorhiza kurroa* Benth
- ✓ To carry out a thorough review on scientific reports on *Picrorhiza kurroa* Benth

2. REVIEW OF LITERATURE

Review of literature was done for the medicinal plants *Picrorhiza kurroa* Benth. A thorough search of literature revealed that there are around four hundred reports on the various scientific studies carried out with the plant *Picrorhiza kurroa* from 1956 to 2017

2.1. Review on *Picrorhiza kurroa* Benth

The available literature on *Picrorhiza kurroa* is 1951 to 2016 is reported in the following sessions.

2.1.1 Isolation of Chemical Constituents from *Picrorhiza kurroa*

2.1.2. Quantification of Constituents of *Picrorhiza kurroa*

2.1.3. Medicinal Potential of *Picrorhiza kurroa*

2.1.4. Patents on *Picrorhiza kurroa*

2.1.5. Other Studies on *Picrorhiza kurroa*

2.1.1. Isolation of Chemical Constituents from *Picrorhiza kurroa*

Isolation of picrorhizin from the rhizomes of *Picrorhiza kurrooa*

Picrorhizin, the bitter principle of *Picrorhiza kurrooa*, was isolated and its structure was characterized as glucosido vanilloyl glucose. From the ether extract the drug a sesquiterpene-like fragrant oil (the odor principle), tripalmitin, and a phytosterol were isolated. (Yeh, Ping-Yuan 1952)

Chemical examination of Picrorhiza kurrooa - Studies on the synthesis of kutkin

Chemical examination of *Picrorhiza kurrooa* gave a new bitter principle, picroside I. Its structure was established as 6'-O-trans-cinnamoylcatalpol.(Rastogi, R. P. 1959)

Natural products from medicinal plants, XXII. Isolation and structure elucidation of a new picroside from *Picrorhiza kurrooa* Royle and Benth

Besides 6'-cinnamoylcatalpol (I) and 6-vanilloylcatalpol, *Picrorhiza kurrooa*. contained a third picroside which was characterized to be 6'-(4-hydroxy-3-methoxycinnamoyl)catalpol. In addition a mixture of 6-cinnamoyl-□-D-glucopyranose and 6-cinnamoyl-□-D-glucopyranose was isolated which presumably was formed by cleavage of 6'-cinnamoylcatalpol during work-up.(Weinges, Klaus; Kuenstler, Klaus 1977)

Natural products from medicinal plants. XXV. Isolation and structure determination of new compounds from the aqueous sodium carbonate extract of *Picrorhiza kurrooa*

From the aqueous sodium carbonate extract of *Picrorhiza kurrooa* ten compounds were isolated after acetylation, as the crystalline acetates. Three of the acetate derivatives were identified and characterized as pentaacetyl-6'-cinnamoylcatalpol, hexaacetyl-6-vaniloylcatalpol and hexaacetylcatalpol by comparison with authentic samples. The structure of seven of the compounds was elucidated by X-ray structural analytical and spectroscopic methods.(20R)-3□,16□,20,25-tetrahydroxy-2□-(-D-tetraacetylglucopyranosyloxy)-10□-cucurbit-5-en-22-one obtained as a crystalline derivative was characterized as a C30-cucurbitacin glucoside. Three acetylated cucurbitacins glucosides identified from the acetylated fraction were found to have a shorter side chain unlike the derivatives isolated from the neutral extract. (Weinges, Klaus et al., 1986)

A process for the isolation of a polysaccharide fraction from *Picrorhiza*

A process is described for the isolation of a novel polysaccharide fraction from *Picrorhiza Kurrooa*, devoid of cucurbitacin, iridoids and their glycosides and having mean molecular weight of 12,000, with significant immunomodulatory and anti-complement properties, is patented(Satti, et al., 2000)

Extraction of picroside I from *Picrorhiza* and the pharmaceutical compositions for the treatment of hepatitis

Extraction of picroside I from *Picrorhize* and the pharmaceutical composition containing *Picrorhize kurroa* is patented for the treatment of hepatitis.(Zhou, Yawei 2006)

A composition and method for preparation of a water-soluble extract of defined content of kutkin from *Picrorhiza kurrooa* rhizomes

A process for obtaining a compound with a defined purity of kutkin contents (i.e., mixture of kutkoside and picrosides) from rhizomes of *Picrorhiza kurrooa* plant by High Performance Liqued Chromatography is patented.(Joshi, Devi Datt 2011)

Synergetic antiviral and immune boosting activity of combinational lipophilic extracts of plants from family Scrophulariaceae and Glycyrrhiza glabra from Fabaceae family

From the underground parts of *Picrorhiza kurroa* tree, new caffeoyl glycosides and glycosides together with caffeic acid derivatives were isolated. (Kumar et al., 2010)

Isolation of a cucurbitacin from *Picrorhiza kurroa* by column chromatography and its characterization

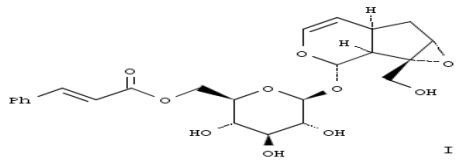
A simple and convenient column chromatographic method was developed to isolate cucurbitacin (aglycon part of cucurbitacin glycoside) from the commercial extract of ***Picrorhiza kurroa*** and its structure was confirmed by chemical and spectral studies. A reversed phase HPLC of the commercial extract using gradient solvent system indicated the presence of 20 components. **(**Nair, Sindhu et al.,2011)

Isolation and characterization of picroside I and kutkoside from *Picrorhiza kurrooa* Benth and simultaneous quantification using HPTLC

Two irridoid glycosides, picroside I and kutkoside were isolated and their content in ***Picrorhiza kurrooa*** was determined by a modified HPTLC method. (Dharmender, Rathee et al., 2011)

Isolation and purification of picroside-I and picroside-II

A process for isolation and purification of picroside-I (I) and picroside-II (II) from *Picrorhiza* plant by reverse phase chromatography is patented. (Sood et al., 2014)



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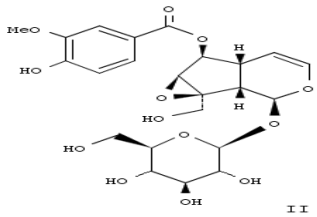


II

A process for the isolation of a polysaccharide fraction from *Picrorhiza Kurrooa*

A process is described for the isolation of a novel polysaccharide fraction from *Picrorhiza Kurrooa*, devoid of cucurbitacin, iridoids and their glycosides and having mean molecular weight of 12,000, with significant immunomodulatory and anti-complement properties, is patented

Method for extracting, separating and purifying picroside I and picroside II from *Picrorhiza*

A method for extracting separating and purifying picroside I and picroside II from *Picrorhiza Kurrooa* Royle ex Benth or *Picrorhiza scrophulariaeflora Pennell* by reversed phase column chromatography is patented.The purity of the compound is greater than 90%.

A composition and method for preparation of a water-soluble extract of defined content of kutkin from *Picrorhiza kurrooa* rhizomes

A process for obtaining a compound with a defined purity of kutkin contents (i.e., mixture of kutkoside and picrosides) from rhizomes of *Picrorhiza kurrooa* plant by High Performance Liqued Chromatography is patented.

Synergistic antiviral and immune boosting activity of combinational lipophilic extracts of plants from family *Scrophulariaceae* and *Glycyrrhiza glabra* from *Fabaceae* family

From the underground parts of *Picrorhiza kurroa* tree, new caffeoyl glycosides and glycosides together with caffeic acid derivatives were isolated.

Method for preparing total iridoid glycoside extract of *Picrorhiza kurrooa*

Extraction of a hepato protective fraction containing picroside I and kutkoside, from *Picrorhiza kurrooa* is patented (Aswal *et al.,* Patent, 1992**)**

This invention provides a method of preparing total iridoid glycoside extract of *Picrorhiza kurrooa*. The total iridoid glycoside extraction of *Picrorhiza kurrooa* is isolated by extracts with organic solvent and purified by liqued chromatography on polystyrene type macroporous adsorbent resin column or other methods. The iridoid glycoside can be used for treatment of liver diseases (Wang, Jianzhong and Wang, Junmin, Patent, 2003)

Extraction of picroside I from *Picrorhiza* and the pharmaceutical compositions for the treatment of hepatitis

Extraction of picroside I from *Picrorhize* and the pharmaceutical composition containing *Picrorhize kurroa* is patented for the treatment of hepatitis (Faming Zhuanli Shenqing, 30 pp., Patent, 2006)

Chemoprofiling and tissue culture studies on *Picrorhiza kurroa* Royle ex Benth. for production of picroside II

An ultrafast flash chromatographic method was also developed for isolation of picroside-II from extracts of *Picrorrhiza kurroa* (Rehman *et al.,* 2014)

2.1.2. Quantification of Constituents of *Picrorhiza kurroa*

376. Colorimetric estimation of total iridoid content of *Picrorhiza kurrooa*

A colorimetric method has been developed for the selective analysis of the total iridoid content of the rhizomes of *Picrorhiza kurrooa* in terms of catalpol. The method of analysis is based on the reaction between iridoid compounds and primary amine. The method was validated as per the ICH guidelines for linearity, accuracy and precision. Several other rhizome samples of the plant were also assayed using this method. The method developed is precise, sensitive, reproducible and easy to perform and can be used for the standardization of crude drug (Narayanan Pushpa and Akamanchi, 2003).

HPTLC Quantification

Development and quantification of HPTLC method for the estimation of kutkin in *Picrorhiza kurroa* (Jadhao, et al., 2009)

A simple and reproducible high performance thin layer chromatographic method was developed and validated for the estimation of kutkin in *Picrorhiza kurroa*. The stationary phase used was precoated silica gel 60F254. The solvent system of chloroform: methanol (8.5:1.8, vol./vol.) was used as mobile phase, the detection of spot was carried out at 258 nm (Jadhao *et al.,*2009)

HPTLC analysis of rhizomes of *Picrorrhiza kurroa* and Aarogyawardhini bati(Mohammed Rageeb, et al., 2012)

Arogyavardhini bati is an ayurvedic formulation containing 50% *P. Kurroa* extract and is used for treatment of fever, diabetes, obesity, skin diseases, and liver diseases. The presence of *P. kurroa in* the Arogyawardhini bati was confirmed through HPTLC fingerprinting of the formulation and compared with the authentic ingredients. It was observed that the chromatographic analyses complement each other in their findings and can be used effectively for the identification of the raw materials in Aarogyawardhini bati (Mohammed *et al.,*2012)

TLC densitometric quantification of picrosides (picroside-I and picroside-II) in *Picrorhiza kurroa* and its substitute Picrorhiza scrophulariiflora and their antioxidant studies

A simple and precise HPTLC method was established for the simultaneous determination of picrosides (picroside-I and picroside-II) in two different *Picrorhiza* species*, P. kurroa and P. scrophulariiflora*. Separation and quantification was achieved by HPTLC with chloroform-MeOH (88:12, vol./vol.) as mobile phase and using pre coated silica gel 60F254 aluminum plates (Tiwari *et al.,* 2012).

Development of a validated HPTLC method for quantitation of phyllanthin, hypophyllanthin and picroside-II in marketed herbal formulation (Valiliv capsules)

A rapid and sensitive high-performance thin-layer chromatographic (HPTLC) method was developed and validated for quantitative estimation of Picroside-II, Phyllanthin and Hypophyllanthin simultaneously in the marketed formulation (Vallilivcapsule) containing *Picrorhiza kurroa* and *Phyllanthus niruri*. The sample (Vallilivcapsule) was chromatographed on silica gel 60F254- TLC plates, with Hexane: Ethyl acetate(6:3 vol./vol.) solvent system and scanned at 278nm (Sheikh *et al.,* 2013).

Chemoprofiing and tissue culture studies on Picrorhiza kurroa Royle ex Benth. for production of picroside II

High performance thin layer chromatographic (HPTLC) method was developed for estimation of picroside II in *Picrorhiza kurroa* samples collected from different places of India. The separation was achieved with chloroform: methanol: formic acid(8:2:0.1, vol./vol./v) as solvent system on precoated silica gel 60 F254 TLC plates. The densitometric quantification of picroside II was carried out at wavelength 265 nm, giving well resolved peak of picroside II at r_f 0.38± 0.2. In vitro tissue culture of *Picrorrhiza kurroa* was also carried out. The callus cultures and regenerated cultures with embryogenic calli were developed and maintained up to 220 days. The tissue cultured samples were tested for their potential for production of picroside, which was quantified by the validated HPLC method (Rehman *et al.,* 2014)

HPTLC Assisted Evaluation of Picroside Content in the Accessions of *Picrorhiza kurrooa* Grown at Different Altitudes in Nepal

Picroside-I and kutkoside are the bioactive marker metabolites of kutki. 27 Samples of *P. kurrooa* growing at different altitudes (3829-4295m) in Western Development Region and Central Development Region of Nepal were screened for picroside-I and kutkoside content. The rhizomes were shade dried, milled, defatted, and extracted with hot methanol. Separation and quantification of picroside-I and kutkoside was achieved on pre coated silica gel 60 F254 aluminium plates with mobile phase chloroform-methanol (75:25, vol./vol.) The r_f of picroside-I is 0.55 and r_f of kutkoside is 0.41. The picroside -I and kutkoside content ranged from 0.61-5.28% and 0.79-9.47%, respectively. Seven chemically superior *P. kurrooa* plant samples collected from the different locations and containing high kutkin content were identified for their conservation and cultivation (Sah *et al.,* 2015).

Reproducible reversed-phase high-performance thin-layer chromatography-based quality-control method for the endangered medicinal plant *Picrorhiza kurroa* Royle ex Benth

A new reversed phase-high performance thin layer chromatographic (RP-HPTLC) method for quantitative assessment of picrosides in leaf and rhizomes of Picrorhiza kurroa was developed. Shade-dried leaves and rhizomes samples of *Picrorhiza kurroa* were extracted with methanol and analyzed for picrosides. The solvent system methanol-water-formic acid showed good separation of picrosides I and II (Kumar *et al.,* 2015)

HPLC Quantification

Phytochemical analysis of rhizomes and roots of medicinal plant *Picrorhiza kurrooa* grown at different Altitudes

Picrotin and picrotoxin contents in rhizomes and roots of *Picrorhiza kurrooa* was analyzed by HPLC. Changes in active constituents was noted in *P. kurrooa* when grown at different altitudes (Kumar and Kumar,2005).

Simultaneous determination of sugars and picrosides in Picrorhiza species using ultrasonic extraction and high-performance liquid chromatography with evaporative light scattering detection

High altitude growing plants are shown to have high levels of sugars to enhance their tolerance to abiotic stresses such as drought and freezing temperatures. A simple, sensitive, selective and reliable HPLC method based on ultrasonic extraction and evaporative light scattering detection (ELSD) has been developed and validated for the simultaneous determination of sugars (xylose, xylitol, mannitol, glucose and sucrose) and picrosides (picroside-I and picroside-II) in two species *Picrorhiza kurroa* and *P. scrophulariiflora*. The analysis was carried out on a Zorbax amino column (250 mm × 4.6 mm i.d., 5 □m) with isocratic elution of acetonitrile:water (78:22, vol./vol.) (Bhandari *et al.,* 2008)

Online HPLC-DPPH method for antioxidant activity of *Picrorhiza kurroa* Royle ex Benth. and characterization of kutkoside by Ultra-Performance LC-electrospray ionization quadrupole time-of-flight mass spectrometry

The free radical scavenging effect of *P. kurroa* was assessed by comparative HPLC-DPPH and colorimetric DPPH methods. The study revealed that colorimetric method showed very less free radical scavenging effect while HPLC-DPPH method showed high activity, The quantity of kutkoside, an important ingredient of a potent hepatoprotective formulation "kutkin/ picroliv" was also analysed by ultra-performance liquid chromatography coupled with diode array detection/electrospray ionization quadrupole time-of-flight mass spectrometry (UPLC-DAD/ESI-QTOF-MS). Earlier reports characterized kutkoside (picroside-II) as a single compound, however, the present investigation proposed that kutkoside is a mixture of iridoid glycosides namely, picroside II, picroside IV and 6-ferulloylcatalpol (Bhandari *et al.,* 2010)

HPLC-based quantification of picroside-I and picroside-II in Picrorhiza kurroa and its population dynamics in Kashmir Himalaya

Plant samples of *Picrorhiza kurroa* were collected from the alpine and sub-alpine areas of Kashmir Himalaya (33°-36° N and 72°-80° E) for chemo-profiling using HPLC analysis. The HPLC analysis revealed higher content of picroside-I (5.65%) in plants collected from Lidderwas-Sonamarg region and a lower level (4.16%) in the plants collected form Daksum Range-Kokernag. However, reverse was true for picroside-II. Picroside-II content was higher than picroside-I content at both the locations (Ashraf *et al.,*2011)

Development and validation of RP-HPLC method for simultaneous estimation of picroside I, plumbagin, and Z-guggulsterone in tablet formulation

A reversed-phase high-performance liquid chromatography method for the simultaneous estimation of picroside I, plumbagin, and Z-guggulsterone in a polyherbal formulation containing *Picrorhiza kurroa, Plumbago zeylanica, and Commiphora wightii* extracts was developed and validated. The analysis was performed on a C18 column using the mobile phase consisting of solvent A (acetonitrile) and solvent B (0.1% orthophosphoric acid in water) with the following gradient: 0-12 min, 25% A; 12-17 min, 25-80% A; 17-32 min, 80% A; and 32-37 min, 80-25% A at a flow rate of 1 ml/min. Ultraviolet detection was at 255 nm (Akhade *et al.,*2013)

2.1.3. Medicinal Potential of *Picrorhiza kurroa*

Anti oxidant Activity

Free radical scavenging potential of Picrorhiza kurrooa Royle ex Benth

The antioxidant activity of *Picrorhize kurrooa* extract was studied by lipid peroxidation assay using rat liver homogenate phospholipids. Besides, reduced glutathione showed very encouraging activity. The results augment the wide use of plant in the indigenous system of medicine, which may partly be due to antioxidant and free radical scavening activity of the extract (Govindarajan *et al*., 2003)

Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants

Total antioxidant capacities of 133 Indian medicinal plant species including *Pikrorhiza kurroa* was assessed by ABTS, DPPH and FRAP assays, and their total phenolic contents measured by Folin-Ciocalteu assay. These species exhibited a broad range of antioxidant activities, varying from 0.16 to 500.70 mmol TEAC/100 g DW in the ABTS assay. The antioxidant activity values similarly varied with the DPPH and FRAP assays. (Surveswaran *et al*., 2007)

ESR spectroscopy investigation of antioxidant activity and protective effect on hydroxyl radical-induced DNA damage of enzymatic extracts from Picrorrhiza kurroa

DPPH radical-scavenging activity of the pancreatic trypsin and amylo glucosidase extracts from *Picrorhiza kurroa* was highest among protease and carbohydrase extracts and 50% inhibitory concentration (IC_{50}) values were 35.58 and 29.03 micro g/mL respectively (Choi *et al*.,2008)

***In-vitro* anti bacterial activities of *Picrorhiza kurroa* rhizome extract using agar well diffusion method**

The antimicrobial potential of acetone, ethanol, methanol, aqueous and hexane extracts of rhizome of *Picorhhiza kurroa* against selected bacterial strains was analyzed (Vinoth Kumar *et al*.,2010).

Microwave solvent extraction (MSE) as an effective technique against traditional solvent extraction (TSE) for screening different plant extracts for antioxidant activity

Eighteen plants belonging to 12 families were evaluated for their in vitro antioxidant activity, *in vitro* antioxidant activity by DPPH assay, superoxide anion radical scavenging activity and total phenolic content (TPC). Extracts were prepared by microwave extraction and by traditional solvent extraction method. The results indicate that extracts prepared by microwave extraction showed potent antioxidant activity in comparison to the extracts prepared by traditional solvent extraction **(**Mathur *et al*.,2011**).**

Characterization of antioxidant activity of agueous extract of twenty selected medicinal plants growing in north-western Himalaya

The phenolic contented antioxited potential of agues extracts of roots of *Picrorhize kurroa* along with 19 plants in colorimetry the aguese extracts of *Picrorhize kurroa* total phenolic contants 18.67±0.17 (mg GAE/g) DPPH % 60.84±0.01 FRAP 132.73±2.65 (Guleria *et al*., 2011).

Evaluation of antioxidant activity of *Picrorhiza kurroa* (leaves) extracts

The ethanol, ethyl acetate and butanol extracts of **of leaves *of Picrorhiza kurroa* and** luteolin-5- O-glucopyranoside and picein isolated from butanol extract were evaluated for assay antioxidant activity by, 2,2-diphenyl-1- picrylhydrazyl radical and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay (Kant *et al*.,2013).

***In vitro* antioxidant activity and phenolic contents in methanol extracts from medicinal plants**

The antioxidant activity of *Picrorhize kurroa* root extract was analysed along with 250 other plants. Quantitative and qualitative analysis of phenolic compounds of the extracts was also done by RP-HPLC method. The total phenolic content of the 80% methanol extracts roots of the *Picrorhize kurroa* was found to be 60.31± 1.32 mg GAE per g of dry sample. DPPH redical scavenging activity 4.16±0.13 and FRAPV 279.5±9.32 fe(II) per g dry weight (Guleria *et al*., 2013).

Screening of free radical scavenging activity of Arogyavardhini vati

Arogyavardhini vati is a potent formulation explained in rasashastra for maintaining health. *Picrorhiza* kurrroa is one of the constituents of this formulation. Antioxidant activity of the formulation was comparable to that of standard ascorbic acid (Sarashetti *et al*., 2013).

Antioxidant and macromolecule damage protective effects of *Picrorhiza kurroa* Royle ex Benth

The iridoid glucosides picroside I, picroside II, picroside III, picroside IV, kutkoside, pikuroside and flavonoids apocynin and vanillic acid identified to be present in the 70% hydroalcoholic fraction of *Picrorhiza kurroa* exhibited DPPH radical scavenging and metal chelating activities with IC50 of 75.16 ± 3.2 and 55.5 ± 4.8 micro g/mL respectively (Krupashree *et al*., 2014)

Hepatoprotective Activity

Hepatoprotective activity of kutkin - the iridoid glycoside mixture of Picrorhiza kurrooa

Kutkin, the iridoid glycoside mixt. (containing mainly kutkoside and picroside I) extracts from the root and rhizome of *Picrorhize kurrooa,* exhibited hepatoprotective activity against liver damage induced by galactosamine in rats and against damage induced by Plasmodium berghei in mastomys(Ansari etal., 1988)

Hepatoprotective effects of Artemisiae capillaris Herba and Picrorrhiza Rhizoma combinations on carbon tetrachloride-induced subacute liver damage in rats

The hepatoprotective properties of *Artemisiae Capillaris Herba* (AC) and *Picrorrhiza Rhizoma* (PR) are well known. The aim of this study was to determine the optimal composition of AC and PR mixturs for better complimentary or alternative regimens in reducing the level of hepatic fibrosis.. The AC and PR mixtures showed good synergic hepatoprotective activity that was attributed to increasing free-radical scavenging ability. (Lee et al., 2008)

Formulation and evaluation of proprietary polyherbal formulation for their Hepatoprotective activity

The hepatoprotective activity of a proprietary formulation Hepjaun syrup (HA-I) and modified formulations (HA-II and HA-III) were evaluated and compared statistically after inducing hepatotoxicity in rats.the formulation containing of *Picrorhize kurroa* extracts (Aatel et al., 2010)

Hepatoprotective activity of a polyherbal mixture in carbon tetrachloride and D-galactosamine induced hepatotoxicity in experimental animals

Hepatoproductive effect of the polyherbal mictire containing *Pikrorhize kurroa* assessed sigificent ($p<0.01$) hepatoprotective activity when evident for the total diseege of T 50mg and T100g (Deshpande et al., 2012)

Development and evalution of hepatoprotective herbal formulation. (Plant extract)

The hepatoprotective activity of a poly-herbal formulation containing methanol extract of diferrent parts of the plants, *viz., Eclipta alba* (whole plant), *Picrorrhiza kurroa*(dried rhizome), *Tribulus terristris* (whole plant)*,Nigella sativa*(seeds), *Piper longam*(dried spikes),*terminelia chebula*(immature fruits) was evaluated and tablets were prepared and evaluated for serum marker enzyme and bio-chemical parameters The results indicated that the prepared formulation can be used for liver disorders especially hepatitis, cirrhosis, jaundice, and fatty liver especially. (Vijayabhaskar et al., 2013)

A novel polyherbal formulation with multiple therapeutic effects as antidiabetic, antifatigue, hepatoprotective and antioxidant

A novel polyherbal formulation containing *Picrorhize kurroa* is disclosed comprising natural and herbal constituents devoid of any harmful effects. The formulation has multiple therapeutic effects with antidiabetic and hepatoprotective activity. It also relieves fatigue. It has antioxidant activity which prevents the formation of free radicals. (Rajasekharan et al., 2013)

Hepatoprotective activity of a standardized poly-herbal liver formulation

A poly herbal liver syrup was formulated with lyophilized ethanolic extract of herbs *A. millefolium, C. spinosa, C. intybus and Picrorrhiza kurroa*. The formulation was standardized as per WHO guidelines and assessed for its

hepatoprotective activity (Bigoniya, P; Singh, C.S. 2014)

Efficacy of Hepatoprotective Formulation Picroliv, an active standardized fraction of Picrorhiza kurrooa

Hepatoprotective activity of picroliv against alcohol-carbon tetrachloride induced damage in rat

The hepatoprotective activity of picroliv, the active standardized fraction of Picrorhiza kurrooa, was evaluated against alc.-CCl4 induced liver damage in rat. It showed a significant dose dependent hepatoprotective activity as evidenced by lowering of the elevated levels of liver enzymes. The activity of picroliv was compared with that of silymarin a known hepatoprotective agent.(Tripathi et al., 1991)

Hepatoprotective activity of picroliv isolated from Picrorhiza kurrooa against thioacetamide toxicity on rathepatocytes

Picroliv, a mixt. of the iridoid glycosides kutkoside and picroside I (1.5:1) from the root and rhizome of P. kurrooa, showed dose-dependent protective activity against thioacetamide-induced hepatic damage in the rat. It enhanced the percentage of viable hepatic cells. Picroliv also antagonized the changes in the transaminases and alk. phosphatase produced by thioacetamide both in hepatocytes and in serum. It was more potent than silymarin, a known hepatoprotective agent.(Visen et al., 1991)

Hepatoprotective activity of picroliv, the active principle of *Picrorhiza kurrooa*, on rat hepatocytes against paracetamol toxicity

Picroliv, the active principle an iridoid glycoside maximum isolated from the plant *Picrorhiza kurrooa*, showed dose-dependent (0.75- 12 mg/kg for 7 days) protective activity on isolated rat hepatocytes against paracetamol-induced damage. It increased the viability of hepatocytes and restored the normal activities of GOT, GPT, and alkyl phosphatase in the isolatedhepatocyte suspension as well as in the rat serum. Picroliv was more potent than silymarin, a known hepatoprotectiveagent.(Chander et al., 1992)

Picroliv, picroside-I and kutkoside from *Picrorhiza kurrooa* are scavengers of superoxide anions

Picroliv, the active principle of *Picrorhiza kurrooa*, and its main components which are a maximum of the iridoid glycosides picroside-I and kutkoside were studied in *vitro* as potential scavengers of oxygen free radicals. picroliv, picroside-I, and kutkoside have antioxidant properties which appear to be mediated through an activity like that of superoxide dismutase, metal ion chelators, and xanthine oxidase inhibitors.(Visen et al., 1993)

Prevention of galactosamine-induced hepatic damage by picroliv: study on bile flow and isolated hepatocytes (ex vivo)

Picroliv, a standardized extract from the plant *Picrorhiza kurrooa* containing active constituents, showed a significant dose dependent (3-12 mg/kg p.o. x 7) protective activity against galactosamine-induced hepatic damage in rats. *Picroliv* was found to be more potent than silymarin, a standard hepatoprotective agent.

Anticholestatic effect of picroliv, active hepatoprotective principle of *Picrorhiza kurrooa*, against carbon tetrachloride induced cholestasis

Picroliv showed a dose (3-12 mg/kg, po for 7 days)-dependent choleretic activity, as evidenced by an increase in bile flow and its contents (bile salts and bile acids). Significant anticholestatic activity was also obserwed against carbon tetrachloride induced cholestasis in conscious rats, anesthetized guinea pigs and cats. Picroliv was more active than the known hepatoprotective drug silymarin. (Saraswat et al., 1993)

Hypolipidemic activity of picroliv in albino rats

The hypolipidemic action of picroliv, a standardized prepn. from *Picrorhiza kurrooa,* has been studied in normal as well as in triton- and cholesterol-fed rats. Serum lipids were lowered by picroliv (25 mg/kg b.w.) in triton WR-1339-induced hyperlipemia. The lipid lowering action of the natural product is mediated through inhibition of cholesterol biosynthesis in liver, increased fecal bile acid excretionand enhanced plasma lecithin:cholesterol acyltransferase activity(Khanna et al., 1994)

Protective activity of picroliv isolated from *Picrorhiza kurrooa* against ethanol toxicity in isolated rat hepatocytes

The effect of picroliv, an iridoid glycoside mixture from the roots and rhizome of *Picrorhiza kurrooa,* on ethanol induced toxicity in isolated rat hepatocytes was investigated. The results were compared with other putative hepatoprotective agents such as andrographolide, silymarin, and catalpol.(Visen et al., 1993)

Protective effect of picroliv, active constituent of *Picrorhiza kurrooa,* against oxytetracycline induced hepatic damage

Picroliv, the active constituent of *Picrorhize kurrooa*, showed a dose dependent (1.5-12 mg/kg, orally for 7 days) hepatoprotective activity against oxytetracycline induced hepatic damage in rat. Picroliv was more potent than silymarin a known hepatoprotective drug. (Saraswat et al., 1997)

Curative effect of picroliv on primary cultured rat hepatocytes against different hepatotoxins: an in vitro study

Picroliv, the standardized active principle from the plant *Picrorhiza kurrooa* showed significant curative activity in *vitro* in primary cultured rat hepatocytes against toxicity induced by thioacetamide (200 □g/mL), galactosamine (400 □g/mL), and carbon tetrachloride (3 □l/mL). The results warrant the use of this in *vitro* system as an alternative for in *vivo* assessment of hepatoprotective activity of new agents.(Visen, et al., 1999)

Picroliv - a natural product protects cells and regulates the gene expression during hypoxia/reoxygenation

The protective effect of picroliv (a purified iridoid glycoside fraction from roots of Picrorhiza kurrooa with hepatoprotective, anti-inflammatory and antioxidant properties) was evaluated. The findings suggest that picroliv may act as a protective agent against hypoxia/reoxygenation induced injuries, and the underlying mechanism may involve a novel signal transduction pathway.(Gaddipati et al., 1999)

Picroliv preconditioning protects the rat liver against ischemia-reperfusion injury

The efficacy of picroliv, a potent antioxidant derived from the plant *Picrorhiza kurrooa,* in protecting against hepatic ischemia-reperfusion injury *in vivo.* Was analysed Picroliv. The studies strongly suggest picroliv to be a promising agent for ameliorating injury following ischemia-reperfusion.(Singh et al., 2000)

Comparative efficacy of piperine, curcumin, and picroliv against Cd immunotoxicity in mice

The inhibition of Cd immunotoxicity was analysed with 3 herbal extracts containing piperine-alkaloid, picroliv-glycosides, and curcumin-polyphenols the a curcumin > ameliorative potential was in order of piperine > picrolive(Pathak et al., 2008)

Therapeutic efficacy of Picroliv in chronic cadmium toxicity

The therapeutic efficacy of Picroliv - a standardized fraction of *Picrorhiza kurroa*, was investigated in male rats treated with Cd as CdCl2 (0.5 mg/kg, s.c.) 5 days/wk for 24 wk and Picroliv at 2 doses (6 and 12 mg/kg, orally) was given during the last 4 wk. The hepatic protection by Picrilov is clearly demonstrated in this study while marginal lowering of urinary proteins and enzymes is a pos. signal of the renal protective efficacy of Picroliv, which could be augmented by adopting higher doses and extended regimen. **(**Yadav et al., 2009)

Activity of Iridoids of *Picrorrhiza kurroa*

Inhibitory effect of iridoids on Epstein-Barr virus activation by a short-term in vitro assay for anti-tumor promoters

The in *vitro* anti-tumor promoting effect of the methanolic extracts of iridoids containing three plants and several pure iridoids isolated from other plants has been evaluated.(Kapadia, Govind et al., 1996)

Iridoid glycosides-Kutkin, Picroside I, and Kutkoside from *Picrorrhiza kurroa* Benth inhibits the invasion and migration of MCF-7 breast cancer cells through the down regulation of matrix metalloproteinases

MCF-7 cell lines (Human breast cancer) were used to test whether *Picrorhize kurroa* extracts (PE) and its isolated iridoid glycosides Picroside I (PS), *Kutkoside* (KS), and *Kutkin* (KT) exert the anti-invasion activity via down-regulation of the expression of matrix metalloproteinases (MMPs). The study showed that PE and its isolated iridoids glycosides PS, KS, and KT exhibited considerable cytotoxic potential in a dose-dependent manner.(Rathee et al., 2013)

Herbal Compositions for treating AIDS

Ayurvedic composition for the prophylaxis and treatment of AIDS, flu, TB, hepatitis and sclerosis and the process for preparing the same (Rohatgi, Surendra, U.S., Patent, 1996)

A process for the preparation of an Ayurvedic formulation for treatment of acquired immuno deficiency syndrome (Jain, Prem Chand, Patent, 1999)

A process for the preparation of an Ayurvedic formulation for treatment of AIDS is described. The formulation contains 0.6g of *Picrorhize kurroa* (Jain, 1999, Patent)

Invention of herbal antiretroviral agent against HIV infection / AIDS (

Sangar, et al., 2006)

A herbal anti retroviral agent comprising *Picrorhize kurroa* is prepared for treatment **against HIV infection and patented (**Sangar *et al.,* 2006, Patent**)**

Herbal Compositions for Skin Disorders

Herbal composition for the treatment of skin disorders and fungal infections of the skin and nails

Herbal composition containing *Picrorhize kurroa* is patented for the treatment of skin disorders and fungal infections of the skin and nails (Shah, Eladevi, Patent, 2001)

Skin preparation for external use/external preparation with antiinflammatory effect for skin (Yamaha, et al., patent,2000)

Chinese medicinal liniment for treatment of skin diseases and its production method

Chinese medicinal a containing *Picrorhize kurroa* Is patented for treatment of skin dieases and its production method (Luo, Xiajing, patent,2014)

Nanometer 'Shexiang Qiying' medicinal preparation

(Yang, Mengjun, Patent, 2002)

Other Formulations containing *Picrorhiza kurrooa*

Polyherbal anti-acne composition having anti-lipase and 5□-reductase inhibition activity

The present invention relates to a polyherbal anti-acne preparetion comprising extracts of Symplocos *racemosa, Salmalia malabarica, Picrorhiza kurroa, Vitex negundo*, *Embelia ribes, Terminalia chebula,* and Terminalia bellerica. The composition not only is shown to have excellent activity against Propionibacterium acnes but also has the added advantage of having anti-lipase and 5□-reductase inhibition activity.(Patil et al., Patent,2012)

Anti cancer, antiviral and immune stimulating activity of Miracle An ayurvedic formulation from natural extracts of Swertia chirata, *Picrorhiza kurrooa* and Echinacea purpurea

A ayurvedic formulation composed of extracts of *triterpenoids, caffeic acid derivaties, feluric acid derivets and other esterified derivs of Swertia chirayita, Echinace*a, and *Picrorhiza* plants. The formulation in patented for in *Picrorhiza kurroa* anti cancer, antiviral and immune stimulation activity. Swertia chirata, *Picrorhize kurroa* and Echinacea purpurea(Rao, M. Ramana, Patent, 2012)

Compositions containing platelet activating factor inhibitor and an antioxidant which interferes with the arachidonic acid cascade

A composition prepared with apocynin, *Picrorhiza kurroa*, lactoferrin *Ginkgo biloba*, bee pollen, mlecithin, and *Fucus vesiculosus*. which interferes with the arachidonic acid cascade and also has antioxidant activity is patented The composition is useful for the treatment or the relief of inflammatory disease, thrombosis, cardiac problems, and/or conditions caused by platelet induced blood clotting(Larkins, Nicholas John, Patent, 2002)

Composition and preparation of cookie-like herbal dietary supplements for lactating womenHerbal laxative preparation/Herbal laxative preparation containing bioactive fraction extracted from Picrorrhiza kurroa

Method of increasing the presence of glutathione in cells

Multi-utility herbal formulation of *Azadirachta* and other plant extracts for farming

A multi-purpose herbal formulation, which prevents the pests occurring in the crop fields and comprises of herbs and herbal extracts Including *Picrorhize kurroa* is patented.

Herbal formulation comprising *Azadirachta indica* extract for wound healing and preparation method thereof

A herbal formulation with highly potent wound healing properties, in humans and animals, consisting of aqueous extract of Azadirachta indica, in a mixture of natural oils along with herbs *viz. Berberis aristata or Berberis vulgaris, Curcuma longa, Glycyrrhiza glabra, Jasminum officinale, Picrorhiza kurrooa, Pongamia pinnata, Rubia cordifolia, Saussurea lappa, Terminalia chebula, Trichosanthes dioica, Capsicum and Stellata wild in well-defined ratios* is patented

A process for the preparation of phytomedicinal composition to increase secretion of mammary glands for lactating mothers is patented Singh et al., pat

Herb-based nutritional composition for correction of metabolic disorders(Shankar, Trivadi Shiva, patent, 2009)

Compositions and methods comprising berberine, alpha lipoic acid (LA), and apocynin, or an isomer or derivative thereof for treating cardiometabolic diseases and disorders(Saleh et al., patant, 2014)

Medicine composition containing *Picrorhize kurroa* is patented **for treating spleen and stomach excessive heat type pancreatitis(Jin, Ke, Patend, 2015)**

A Chinese medicinal preparation containing *Picrorhize kurroa* is patented for treating stomach ulcer and its preparation method(Hou,Derun, Patent, 2015)

3. MATERIALS AND METHODOLOGY

The present work was aimed at the Phytochemical Profiling of *Picrorhiza kurroa which* includes Quantitative and Qualitative Analysis

The methodology adopted for this study comprises of

- ❖ Collection and pre processing of plant
- ❖ Extraction of plant material
    - Soxhlet extraction
    - Cold extraction
- ❖ Orgenoleptic analysis of the extracts
- ❖ Solubility and pH tests
- ❖ Phytochemical screening of extracts
- ❖ Determination of phytochemical content
- ❖ Acid hydrolysis of polar extracts
- ❖ TLC analysis of extracts
- ❖ Fingerprinting of extracts
    - Chromotographic fingerprinting-TLC, HPTLC, HPLC, GC-MS
    - Spectral fingerprinting of extracts-UV,IR,NMR

3.1.Collection of Plant

Picrorhiza kurroa rhizomes were collected from Kerala. The plant material was shade dried and pulverised.

3.2.Extraction of Plant

Extraction of *Picrorhiza kurroa* was done with solvents (polar and non-polar) by Soxhlet Extraction (Hot) and Cold extraction.

Hot Extraction

Method 1

About 250g of the plant was extracted in a soxhlet extractor with hexane for 12 hours. The extract was concentrated. The extract concentrate was collected in pre-weighed beaker. To the residual plant extract ethyl acetate was added and again extracted for 8hours. The sequential extract (1litre) was done with 90% ethanol. The extract were concentrated, yield noted and were stored for further analysis.

Method 2

About 100g of plant material was extracted with hexane for 6 hours. Extract was filtered and concentrated. The remaining plant material was refluxed with 90% ethanol for 6 hours. The extract concentrate was stored for further analysis. The yields were noted.

Cold Extraction

Cold extraction was carried out with hexane, chloroform, ethyl acetate, acetone, ethanol and water. About (1g) leaves were soaked in the respective solvent for 6 hours with frequent maceration. The yields were noted.

3.3.Organoleptic Studies

The colour, appearance, and odour of the extracts of leaves was observed.

3.4.Solubility Studies and pH Test

Solubility of the polar and non-polar extracts of *Picrorhiza kurroa* was tested with solvents chloroform, acetone, ethanol, methanol and water. pH of the polar extracts was recorded in Delux pH meter-101.

3.5.Phytochemical Screening (Haraborne *et al.,* 1973*)*

All the extracts were subjected to phytochemical screening by using standard tests.

Test for Alkaloids

Hager's Test

A fraction of plant extract was treated with few ml of saturated aqueous solution of picric acid and observed for the formation of prominent yellow precipitate.

Test for Flavonoids

Test with Sodium Hydroxide

A small amount of the each extract treated with aqueous NaOH and observed for the formation of yellow orange colour.

Schinoda Test

Sample was dissolved in ethanol, warmed and the filtered. Three pieces of magnesium chips were then added to the filtrate followed by few drops of concentrated HCl. A pink, orange or red to purple colouration indicates the presence of flavonoids.

Test for Carbohydrates

Molisch's Test

Each extracts were dissolved in water and treated with molisch's reagent (alcoholic α-napthol). Concentrated sulphuric acid added along the sides of test tube. The formation of a red or violet colour at the interphase of two layers was a positive test.

Test for Terpenoid

Salkowski Test

The extracts were taken in the test tube and few ml of chloroform was added. Then concentrated sulphuric acid was added along the sides. Reddish brown solution indicates the presence of terpenoids.

Libermann-Buchard Test

A fraction of extract was dissolved in chloroform and treated with acetic anhydride and few drops of concentrated H_2SO_4 . Formation of dark green colour was observed.

Test for Phenolic Compounds

Ferric Chloride Test

A small amount of extract was treated with 5% ferric chloride and observed for the formation of deep blue or black colour.

Lead Acetate Test

A small amount of the extract was treated with lead acetate and the formation of the white precipitate was observed.

Test for Steroids

Libermann-Buchard Test

A fraction of extract was dissolved in chloroform and treated with acetic anhydride and few drops of concentrated H_2SO_4 . Formation of dark pink or red colour showed the presence of sterols.

Test with Concentrated Sulphuric acid

The sample was treated with ethanol and concentrated sulphuric acid and observed the formation of violet blue or green colour.

Test for Saponins

Foam Test

Extracts were shaken with distilled water (0.5 ml). Persistence of foam indicate the presence of saponin.

3.6.Determination of Phytoconsituents

Determination of Total Flavonoid (Ordonez *et al.,2006*)

Total flavonoid content of the extracts was determined by the formation of a flavonoid-aluminium complex. The extracts (0.5 ml of ethanol soluble extracts (1mg/ml)) were mixed with 0.5ml of aluminium chloride (2% in ethanol). The mixture was incubated for 60min at room temperature for yellow colour development. Absorbance was measured at 420nm using UV–VIS spectrophotometer. Total flavonoid content was calculated as quercetin equivalent (mg/g) using the following equation

$$Y = 0.217^{*} X$$

Where,

X= absorbance and Y = quercetin equivalent

Determination of Total Terpenoid

Total terpenoid content was determined by colorimetry. 10 mg of each of the five extracts was individually dissolved in 1 ml of methanol. Then, 100 µl of each of these solutions was mixed with vanillin-glacial acetic acid solution (150 µl, 5% w/v) and perchloric acid solution (500 µl). The sample solutions were heated for 45 min at 60◦C and then cooled in an ice-water bath to the ambient temperature. After the addition of glacial acetic acid (2.25 ml), each sample solution's absorbance was measured at 548 nm, using a UV-visible-light spectrophotometer. Ursolic acid (0.025–0.5 mg/mL in methanol) was used as a standard.

3.7.TLC Examination

TLC Examination of Extracts

Thin layer chromatographic analysis was performed for all extracts with pre-coated TLC plates (5*10cm). Samples of non-polar (PIKU (NP)), and polar (PIKU(P), PIKU (E.A SEQ), PIKU(P SEQ)) extracts were dissolved in ethanol one by one separately and were spotted on the TLC plates manually with a capillary tube. The developing solvent system optimized for the sample is given below,

PIKU(NP) - Petroleum ether (10ml) and ethanol (10 drops)

PIKU(NP SEQ) - Petroleum ether (10ml) and ethanol (10 drops)

PIKU(E A SEQ) - Petroleum ether and ethyl acetate (7:3)

$$R_f \text{ value} = \frac{\text{Distance travelled by compound}}{\text{Distance travelled by solvent front}}$$

3.8.Fingerprinting of Extracts

3.8.1.Chromatographic Fingerprinting

Chemical fingerprinting of the plant secondary metabolites can be performed through different methods, which may include UV, FT-IR, NMR, HPLC, HPTLC, GC-MS or a combination of these techniques. Chromatographic and spectral fingerprints analysis of herbal samples has become the most popular and potent tools for quality control of herbal medicines because of its simplicity and reliability. Finger printing of extracts serve a tool for identification, authentication and quality control of herbal products.

3.8.1.1.HPTLC Fingerprinting of Extracts

CAMAG HPTLC instrument was used for the analysis it worked under the room temperature. Ethyl acetate: formic acid: glacial acetic acid: water in the ratio of 10:1.1:1.1:2.6 was used as mobile phase. 1% ethanolic aluminium chloride was used as spray reagent.

3.8.1.2.HPLC Fingerprinting of Extracts

This also used to separate constituents on the basis of their interaction with solid particles of a tightly packed column and the solvent of the mobile phase. Extracts were analysed by Shimadzu HPLC instrument running under room temperature.

3.8.1.2. GC-MS Fingerprinting

Mass to charge ratio can be calculated from GC-MS for non-polar extract.

3.8.2. Spectral fingerprinting

3.8.2.1. UV-Visible Fingerprinting

The extracts were dissolved in distilled alcohol and their respective spectrums were recorded by Double beam UV- Visible spectrometer. It was calibrated and wavelength was adjusted between 200-800nm. After calibration the pure ethanol was taken in the cuvette and the sample which is dissolved in the same solvent was taken in the other cuvette. The UV spectra were recorded.

3.8.2.2 .FT-IR Spectral Finger Printing

Extracts were analysed using Shimatzu FT-IR spectrometer. Spectrum recorded from 4000 to 750 cm^{-1} MIR region. Different bonds (C–C, C=C, C≡C, C–O, C=O, O–H, and N–H) were identified.

3.8.2.3.^{1}H NMR Fingerprinting of Extracts

^{1}H NMR is primarily related to the magnetic properties of atomic nuclei such as the hydrogen atom, the carbon, and an isotope of carbon. Principle behind is splitting of spectral line of hydrogen by neighbouring groups.

4. RESULTS AND DISCUSSION

4.1 Extraction

Table 1 gives the extractive values of different solvent extracts. Extractive value is expressed as percentage yield of extracts. In cold extraction, higher yield of extract was obtained for the hexane extraction followed by sequential 90% ethanolic extract. This indicates a higher percentage of non polar as well as polar metabolites in the rhizomes of the plant.

Table 1 Extractive Values

Hot extraction (6h)		**Cold extraction (6h)**	
Extracts	**Extractive Value(%)**	**Extracts**	**Extractive Value(%)**
PIKU(NP SEQ)	1.42	PIKU(H)	9.71
PIKU(E A SEQ)	1.38	PIKU(EA)	3.20
PIKU(P)		PIKU(AC)	0.73
PIKU(P SEQ)	1.22	PIKU(CH)	2.17
_	_	PIKU(E)	6.47
_	_	PIKU(W)	8.60

4.2. Organoleptic Studies

Organoleptic studies were done for all extracts. Almost all extracts has fragrant smell and are brown shade in colour except JAFL(P SEQ). All polar extracts has waxy apperence. It can be expressed in below table 2.

Table 2 Organoleptic studies on extracts

Samples	Colour	Appearance	Odour
PIKU(P)	Brown	Wax	
PIKU(NP SEQ)	Dark brown	Semi solid	
PIKU(E.A SEQ)	Dark greenish brown	Wax	
PIKU(P SEQ)	Dark green	Wax	

4.2 Solubility Test

Extracts	Chloroform	Acetone	Methanol	Ethanol	Water
PIKU(P)	Partially Soluble	Partially Soluble	Completely Soluble	Completely Soluble	Completely Soluble
PIKU(NP SEQ)	Completely Soluble	Completely Soluble	Partially Soluble	Completely Soluble	Insoluble
PIKU(E.A SEQ)	Completely Soluble	Completely Soluble	Completely Soluble	Completely Soluble	Insoluble
PIKU(P SEQ)	Partially Soluble	Partially Soluble	Completely Soluble	Completely Soluble	Completely Soluble

Table 3 Solubility of polar and non polar extracts

4.3 pH Test

The pH of the water soluble extracts was tested at room temperature. All extracts were slightly basic in nature.

Table 4 pH of Polar Samples

Samples	pH	Temperature(*c)
PIKU (P)	7.06	30
PIKU (E.A SEQ)	7.04	30
PIKU (P SEQ)	7.02	30

4.4.Phytochemical screening

Colour tests were carried out for the extracts. All of the extracts showed the positive result for terpenoid.

Class of Compounds	**Tests**	**PIKU(NP SEQ)**	**PIKU(EA SEQ)**	**PIKU(P)**	**PIKU(P SEQ)**
Alkaloids	Hager's test	-	+	-	-
Flavonoids	Schinoda test NaOH test	- -	- +	- -	- -
Phenolic compounds	Ferric chloride test Lead acetate test	- -	- -	- -	- -
Steroids	Libermann-Buchard test Salkowski test	- -	- -	- -	- -
Carbohydrates	Molisch's Test	-	-	-	-
Terpenoids	Salkowski test Libermann-Buchard test	+ +	+ +	+ +	+ +

Table 5 Phytochemical screening

4.5 .TLC Examination

The concentrated extracts (PIKU(NP SEQ), PIKU(E.A SEQ), PIKU(P) and PIKU(P SEQ) were dissolved in distilled ethanol and subjected to TLC examination. From the TLC analysis it can be proposed that all the plant extracts contain a number of metabolites. Figure 3-5 represent the TLC chromatograms of plant extracts

Table 6 Designated Codes of Solvent Extracts

S.No	**Extracts**	**Code**
1	Hexane sequential extract of *Picrorhiza kurroa*	PIKU(NP SEQ)
2	Ethyl acetate sequential extract of *Picrorhiza kurroa*	PIKU(E A SEQ)
3	90% Ethanolic extract of *Picrorhiza kurroa*	PIKU(P)
4	90% Ethanolic sequential extract of *Picrorhiza kurroa*	PIKU(P SEQ)

Figure (2)TLC Chromatogram of PIKU (NP)

Figur(3)TLC Chromatogram of PIKU (NP)

Figure (4)TLC Chromatogram of PIKU (P& P SEQ)

4.6.Chromatographic Fingerprinting

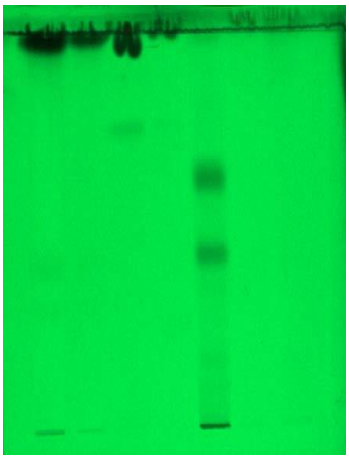
4.6.1 HPTLC Fingerprinting

The extract sample (1mg) dissolved in methanol (10 µl) was spotted on the TLC plates. The solvent system optimised is Ethyl acetate: formic acid: glacial acetic acid: water in the ratio of 10:1.1:1.1:2.6. Ethanolic aluminium chloride (1%) was used as spray reagent. The representation of spots is given below,

Track 1- PIKU(P)

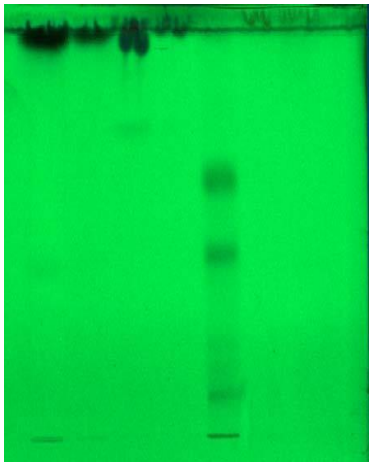
Track 2- PIKU(NP SEQ)

Track 3- PIKU(EA SEQ).

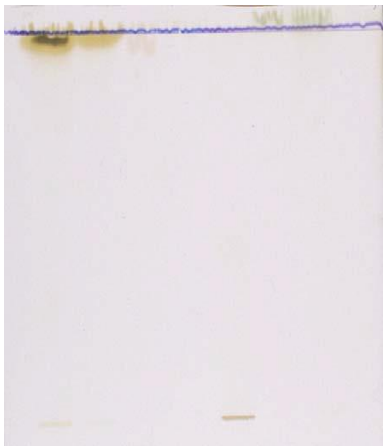


Figure(5) TLC Plate under UV Light at 254 nm

Figure (6)TLC Plate under UV Light at 366 nm



Figure(7) TLC Plate under UV Light at 254 nm with spray reagent



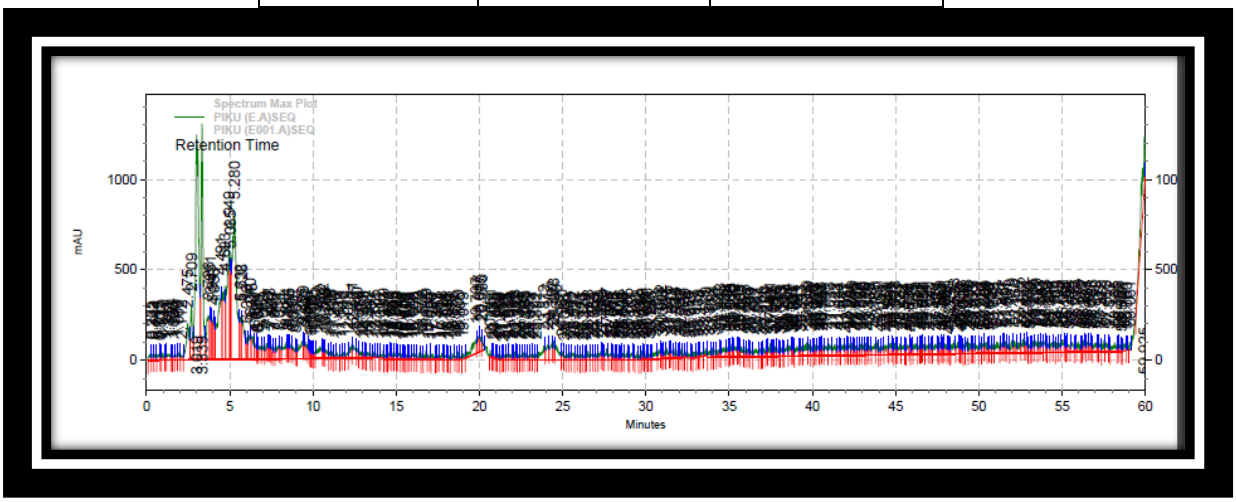
Figure(8) TLC Plate under UV Light with spray reagent

4.6.2.HPLC Fingerprinting

High Pressure Liquid Chromatographic analysis of selected polar extracts (PIKU (E A SEQ) and PIKU (P)) was done the comparison of their chemical profiles. In the chromatogram of ethyl acetate extract of *Picrorhiza kurroa* major peaks were found at retention time (minutes) 9.64, 15.456, 16.555, 29.749 and 38.58 and the polar extract showed major peaks at retention time (minutes)9.557, 17.664, 20.161, 22.176, 23.851, 28.245 and 34.304. Both the extracts showed the two peaks ((retention time (minutes) 38.58 & 23.851)) as major compounds.

Table 8 HPLC Retention Time of Prominent Peaks of PIKU(E A SEQ)

Retention Time(Min.)	Area (%)	Height (%)
2.709	1.222	1.186
3.019	8.790	4.994
3.339	5.501	5232
4.693	2.339	1.626
4.949	3.538	2.548
5.035	1.236	2.127
5.280	7.891	3.203



Figure(7) HPLC Chromatogram of PIKU(E A SEQ)

Table 9 HPLC Retention Time of Prominent Peaks of PIKU(P)

Retention Time(Min.)	Area (%)	Height (%)
11.253	19.379	7.109
11.659	1.264	4.564
11.861	3.440	4.612
12.907	4.425	6.393
13.365	7.188	6.515
14.155	2.042	3.533
14.773	3.512	2,231
15.328	4.722	1.069
17.653	5.129	0.917

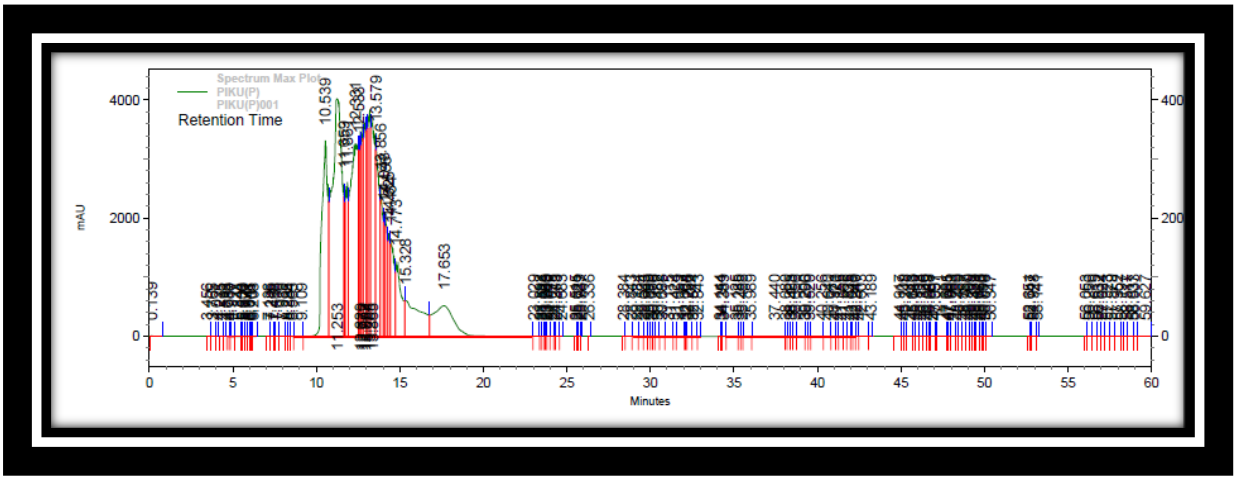


Figure (8) HPLC Chromatogram of PIKU(P)

4.6.3. GC-MS Fingerprinting

The gas chromatogram of PIKU(NP) was recorded. The prominent peaks with area % greater than 5 were noted. The probable compounds were predicted based on NIST library search are listed in table 12

Table 10 GC MS Data and Proposed Compounds in PIKU(NP)

Retention Time(min.)	Area %	Proposed Compounds
18.245	20.696	N-HEXADECANOIC ACID
19.875	42.971	(Z)6, (Z)9-PENTADECADIEN –I-OL
26.573	7.488	TRITETRACONTANE
27.588	6.886	HEPTACOSANE

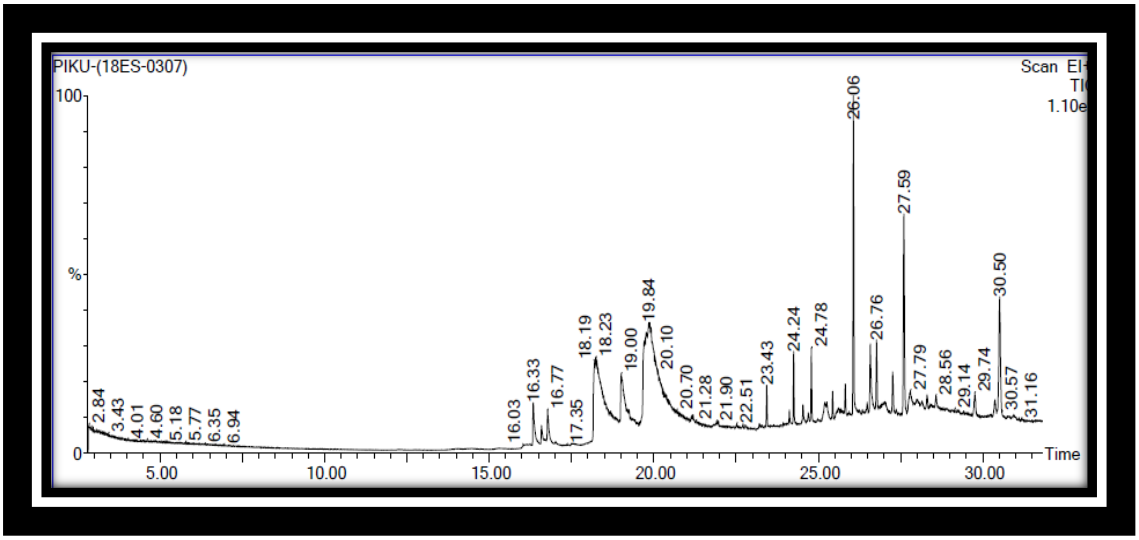
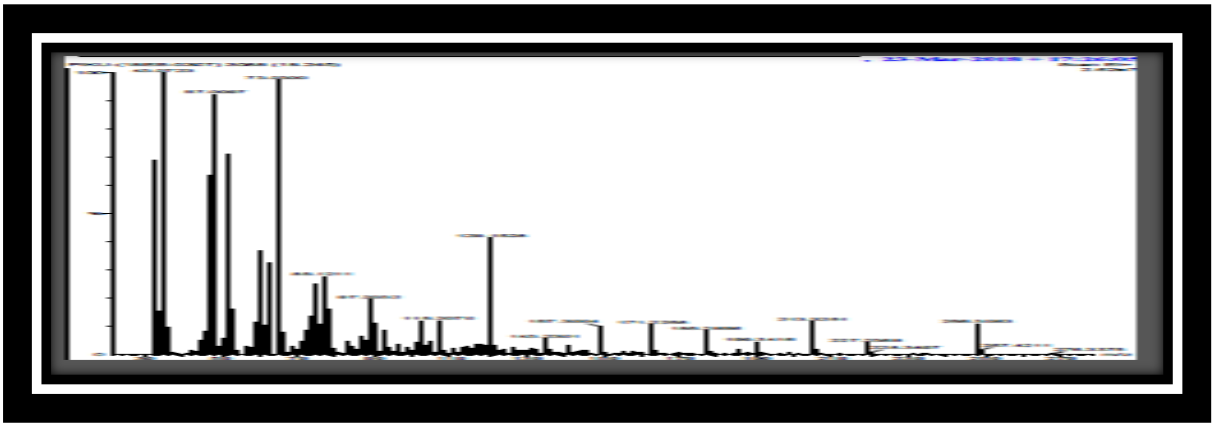
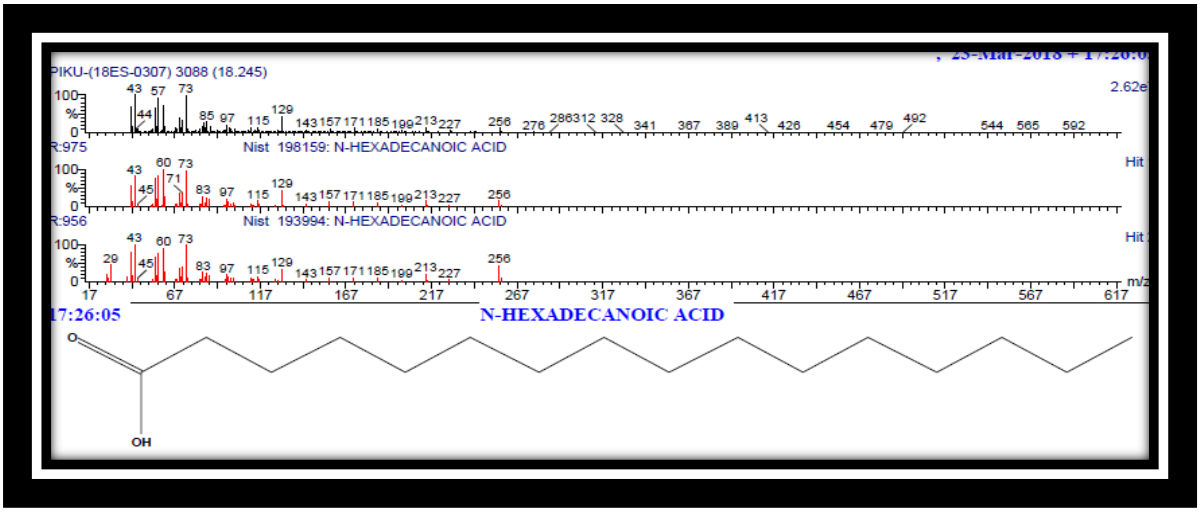


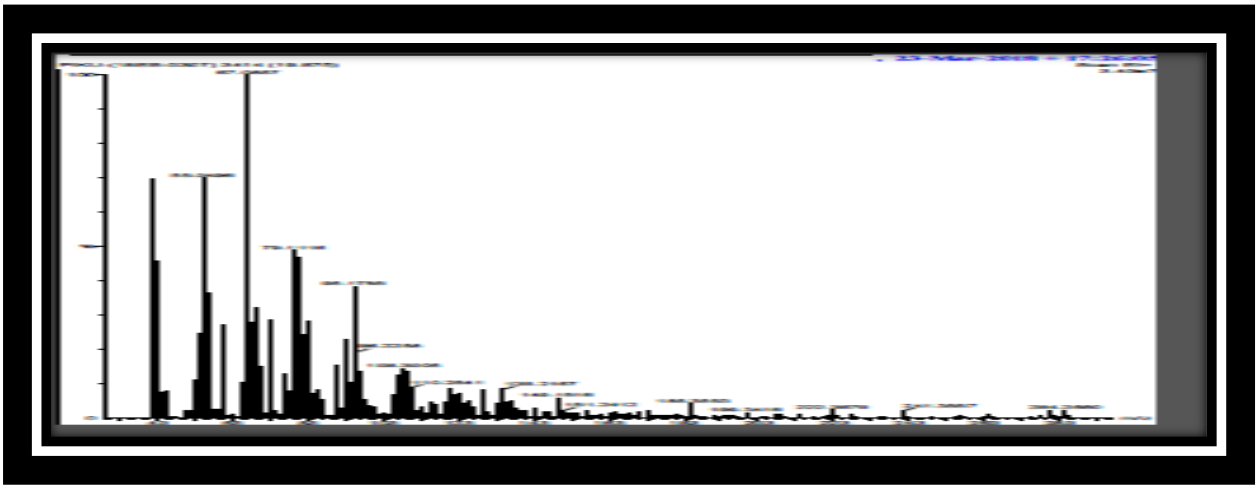
Figure 9 Gas Chromatography of PIKU(NP)



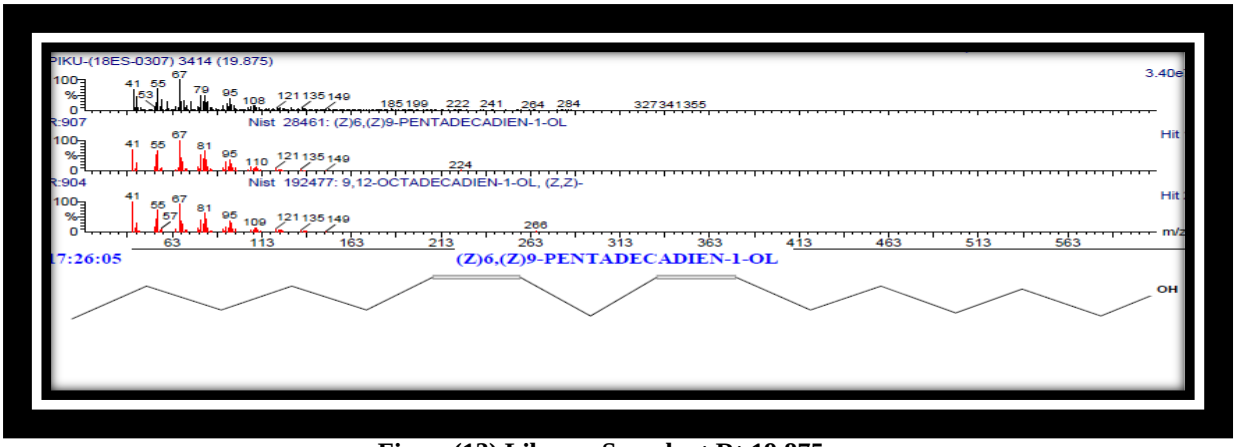
Figure(10) Mass Spectrum Peak at Rt 18.245



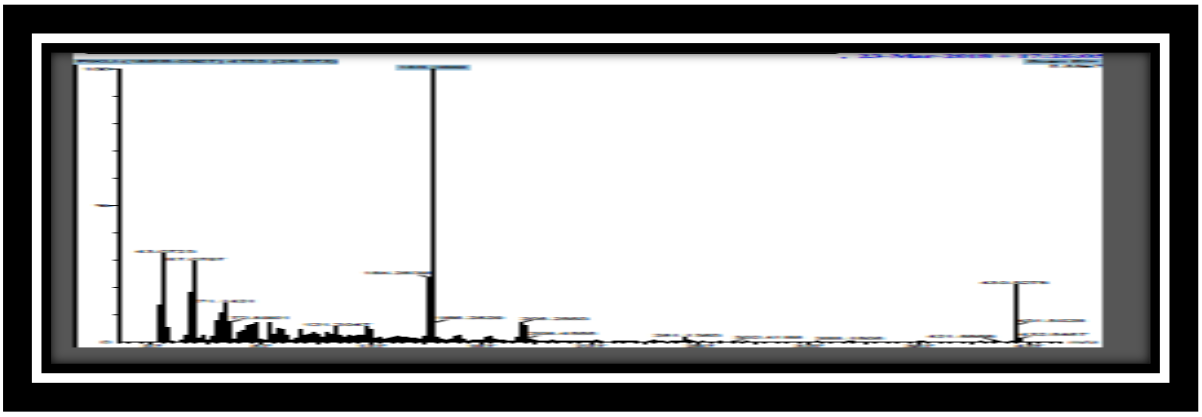
Figure(11) Library Search at Rt 18.245



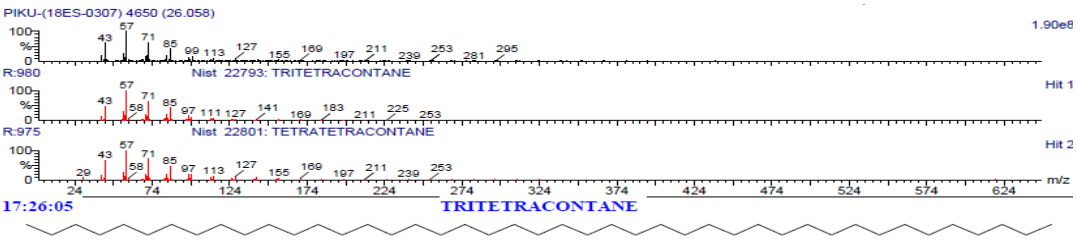
Figure(12)Mass Spectrum Peak at Rt 19.875



Figure(13) Library Search at Rt 19.875



Figure(14) Mass Spectrum Peak at Rt 26.573



Figure(15) Library Search at Rt 26.573

Figure(16)Mass Spectrum Peak at Rt 27.5

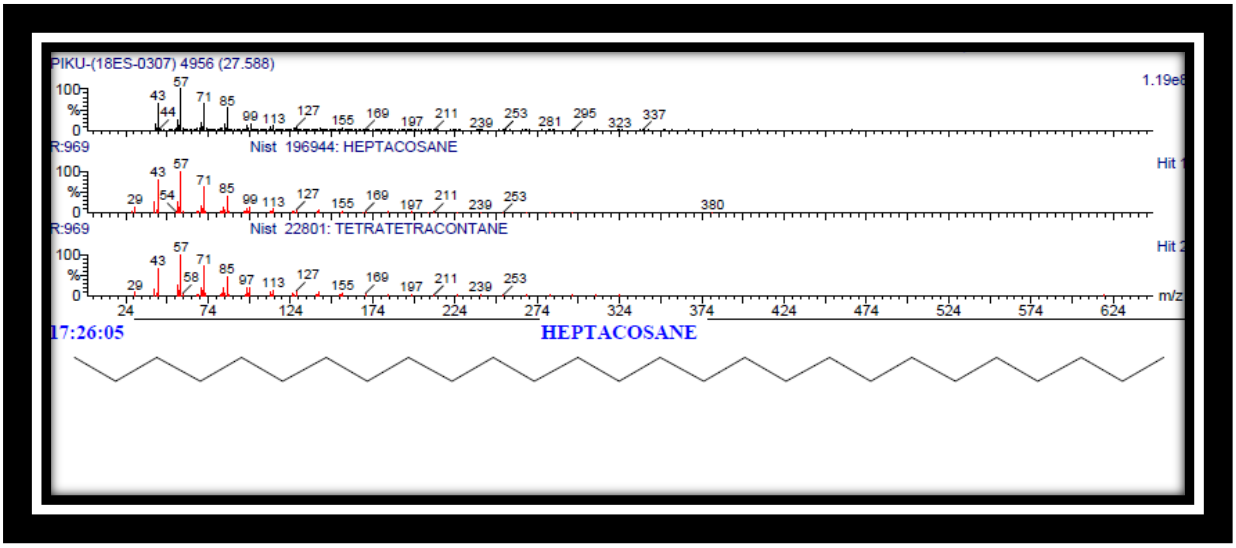


Figure 17 Library Search at Rt 27.5

4.7.Spectral Fingerprinting

4.7.1.UV –Visible Spectrometric Analysis

Represent the UV spectrum of the polar and non-polar extracts. For all extracts shows the visible peak at 665nm corresponds to presence of chlorophyll. The peak at ~420nm may be due to flavonoid and polar groups in the sample.

Addition of sodium ethoxide shift reagent to each of the sample (polar extracts- PIKU(P), PIKU (E A SEQ)) was done to ascertain the presence of flavonoids.

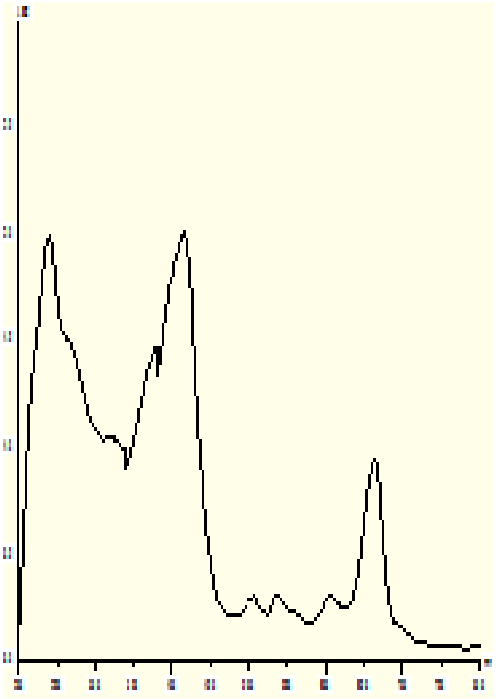


Figure 17 UV Spectrum of PIKU(NP SEQ)

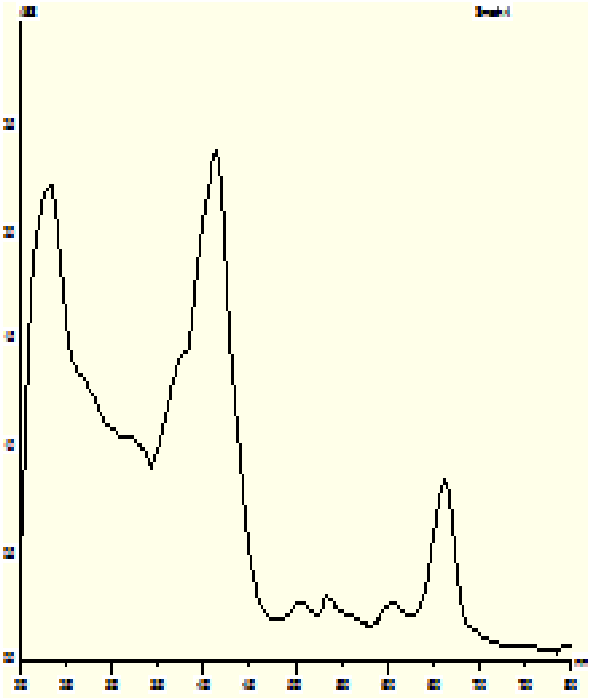


Figure 18 UV Spectrum of PIKU(E A SEQ)

Figure 19 UV Spectrum of PIKU(P)

Figure 20 UV Spectrum of PIKU(P SEQ)

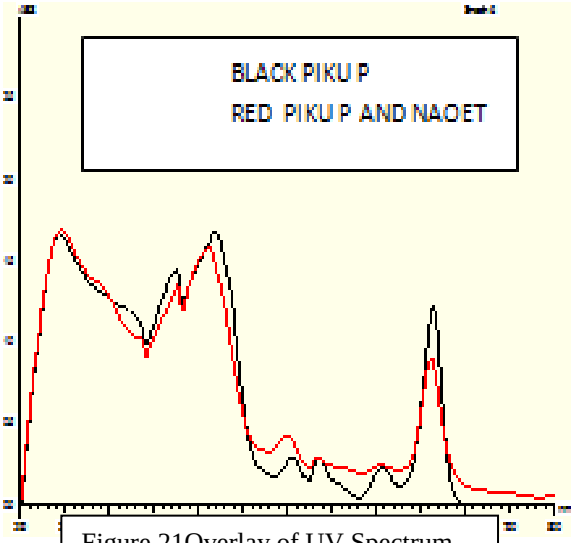


Figure 21Overlay of UV Spectrum of PIKU(P) with UV Spectrum of PIKU(P+NaOEt)

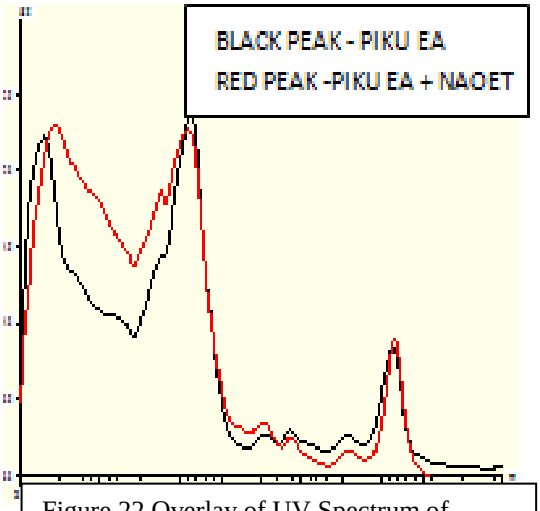


Figure 22 Overlay of UV Spectrum of PIKU(P) with UV Spectrum oPIKU(P+NaOEt)

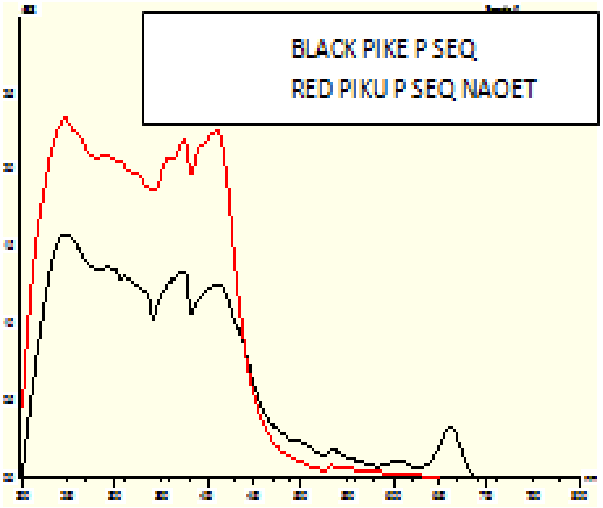


Figure 23 Overlay of UV Spectrum of PIKU (P SEQ) with UV Spectrum PIKU(P+NaOEt)

4.7.2. FT-IR Spectrum of Plant Extracts

Figures (11)show the FT-IR spectrum of the respective solvent extracts. The IR spectrum of JAFL (NP) indicated the presence of non polar constituents only. The peak at 2848 cm^{-1} corresponds to sat urated C-H. Polar extracts shows the characteristic peak at 3388,3344 cm^{-1} , probably due to the presence of alcoholic groups (-OH group). Two more peaks at 2924 and 1716 cm^{-1} showed the presence of C=O functional group.

Table 11 IR Absorption Values of PIKU Extracts

Extracts	**Wave number (cm^{-1})**	**Probable Functional Groups**
PIKU (NP SEQ)	3 2850.79 1735.93 1708.93 1462.04 1381.63,1165.00	OH stretch Alkenes C=O stretch CH_2 group Alcohol(OH) Stretching of C-O
PIKU(E A SEQ)	3363.86 2924.09 2854.65 1716.65 1454.33 1381.03 1165.00 721.38	OH stretch C=O stretch Alkenes C=O Stretch -CH_2 group Alcohol(OH) Stretching of C-O Alkenes(=CH bend)
PIKU(P)	33336.85 2974.23 1697.36 1631.78 1280.73 1161.15	OH stretch C-H stretch C=O stretch Alcohols Carboxylic acid(C=O) O-H stretch

PIKU(P SEQ)	3356.14 2978.09 1620.21 1381.03 1045.42,8255.53	OH stretch Alkenes C=O stretch Alcohol(OH) Stretching of C-O

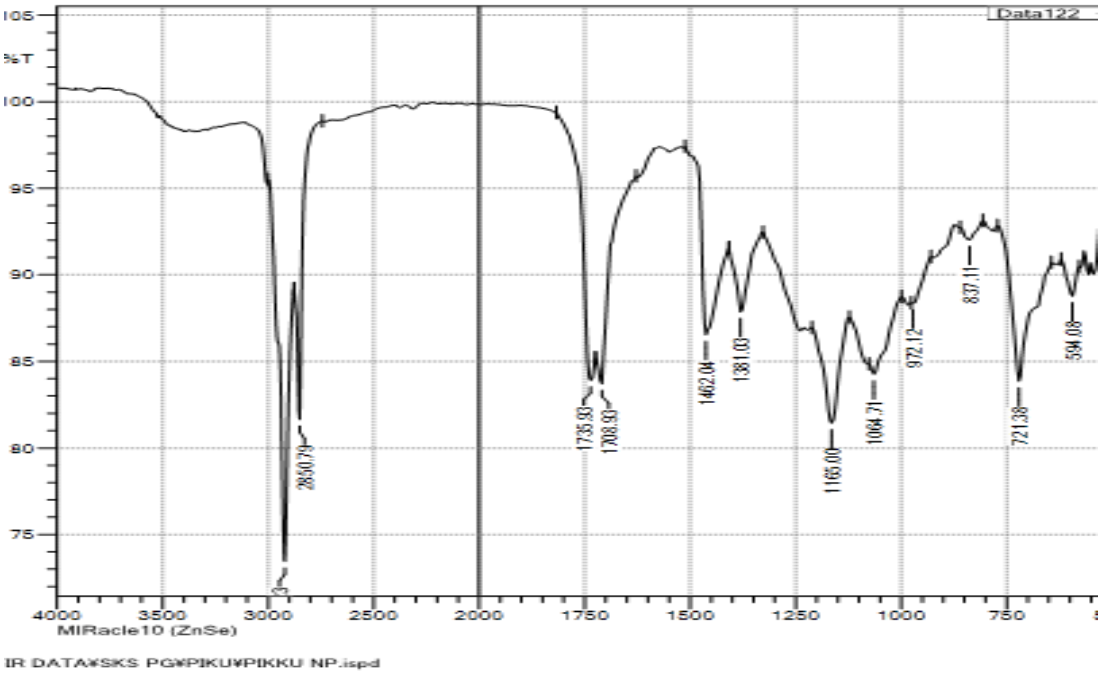


Figure 24 IR Spectram of PIKU(NP SEQ)

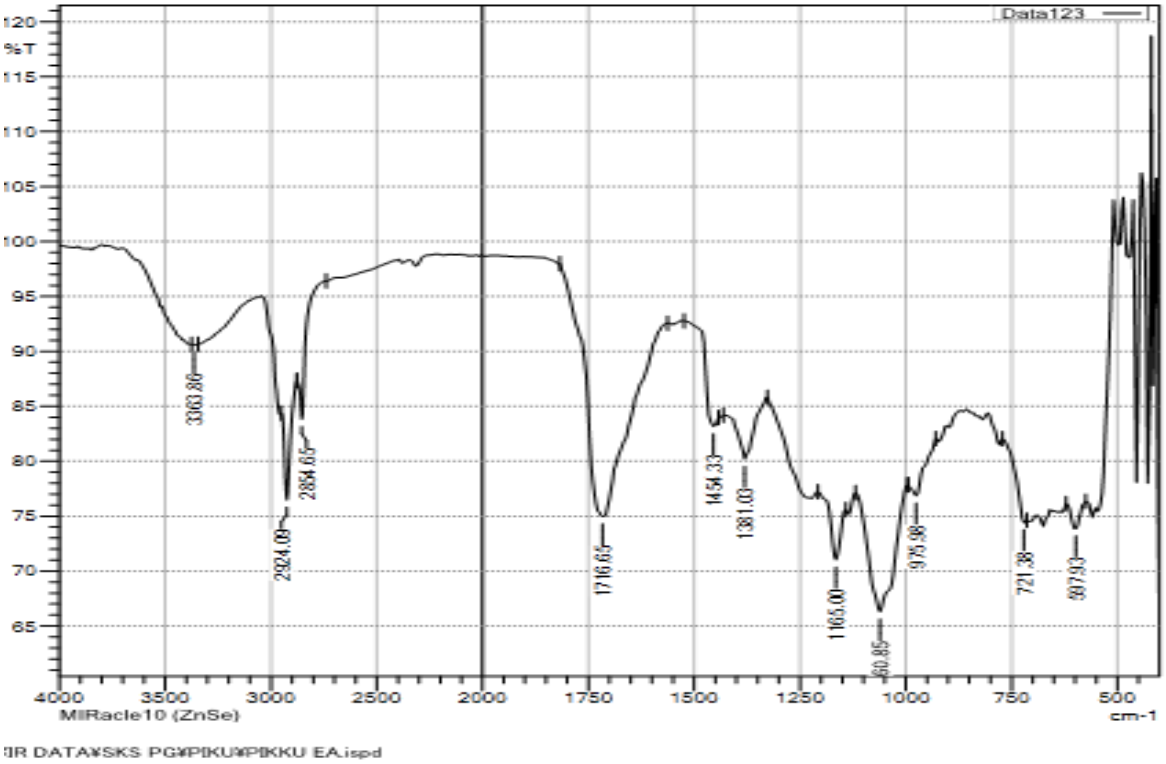


Figure 25 IR Spectram of PIKU(EA SEQ)

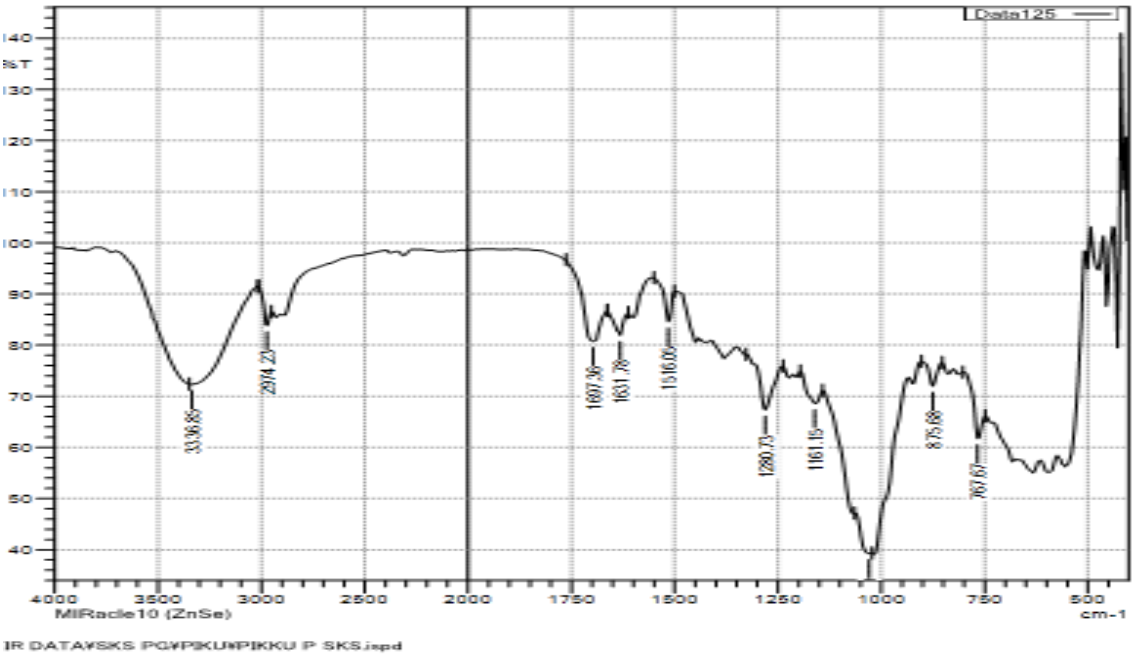


Figure 25 IR Spectram of PIKU(P)

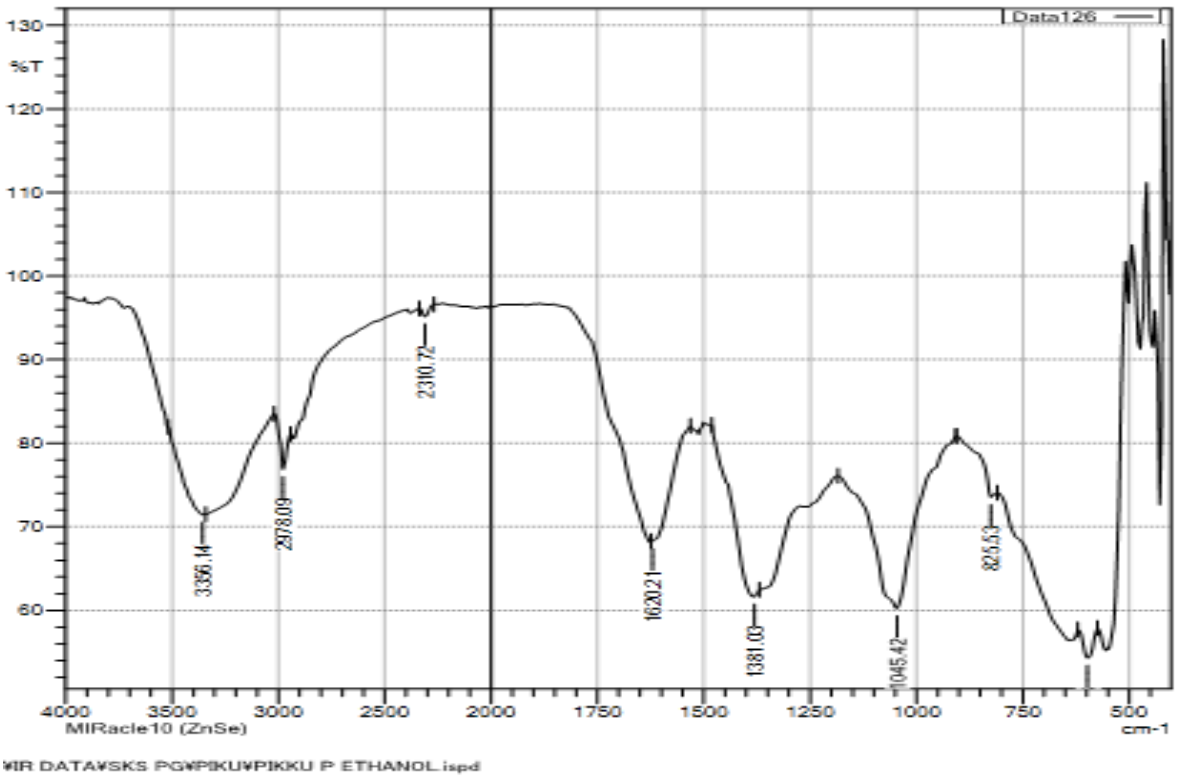


Figure 26 IR Spectram of PIKU(P SEQ)

4.7.3.^{1}H NMR Examination of the chosen extracts

The NMR spectra of solvent extracts of *Picrorhiza kurroa* were recorded to observe the presence of probable groups and their characteristic peaks are summarised in the below table(11)

Table(11) **^{1}H NMR Characteristicas of Extrach**

Extracts	**ppm**
PIKU(NP)	5.36,5.12,4.72,4.66,4.60,4.58 4.28,4.17,4.11,4.05,3.90 2.34,2.32,2.10,2.08,2.06 2.04,2.00,1.68,1.60,1.31,1.25
PIKU (E A SEQ)	7.94,7.87,7.81 5.26,5.24 4.43,4.10 3.80,3.59,3.41,3.40 2.50,2.49 1.50,1.22,1.17,1.10,1.08
PIKU (P)	7.97 7.60,7.52,7.42,7.35 6.84,6.48,6.44,6.30,6.23 5.17,5.04,4.96,4.75,4.72,4.65 1.55,1.17,1.07,1.05,1.02,1.80
PIKU (P SEQ)	7.81 3.86, 3.48 3.15 2.56,2.50,2.49,2.48,2.42,2.17 1.39,1.36,1.16,1.05,0.92,0.83

The presence of saturated protons was indicated by the peaks in the NMR spectrum of non polar extract. Flavonoidal and glycosidic signals are seen in the NMR spectrum of the polar extracts.

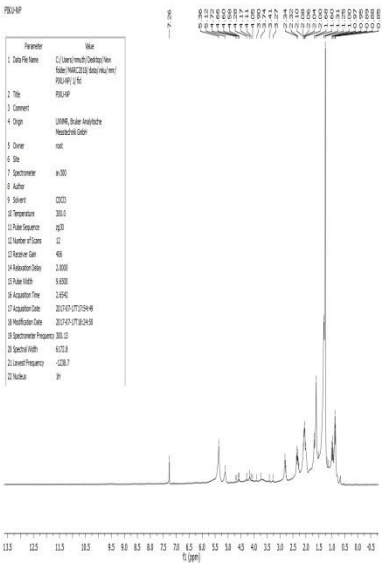


Figure 28 [1]H NMR Spectrum of PIKU(NP SEQ)

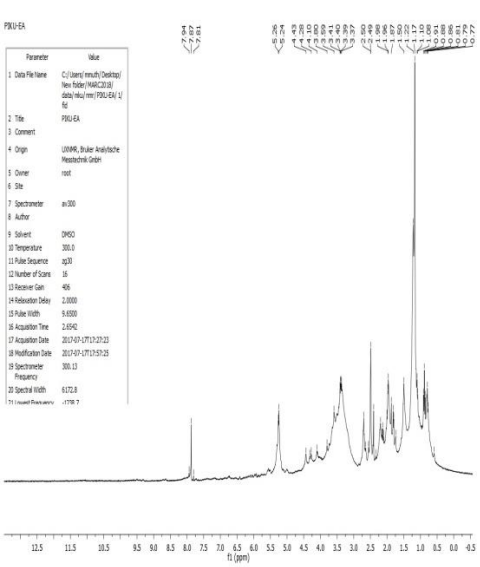
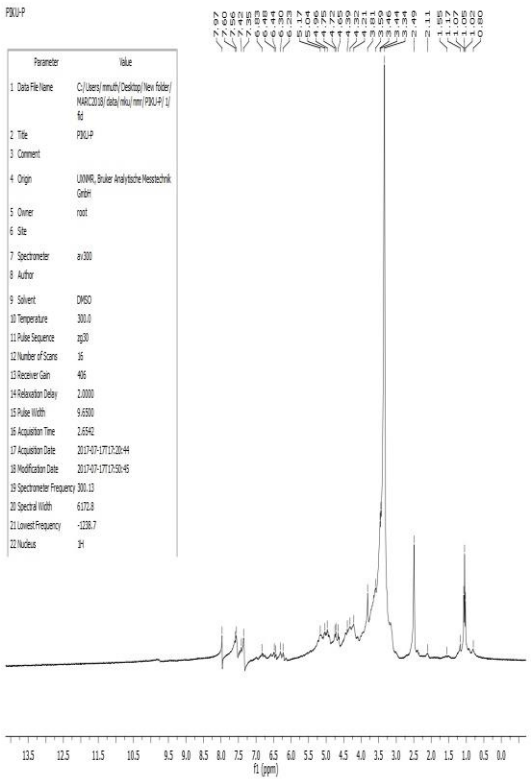


Figure29[1]H NMR Spectrum of PIKU(EA SEQ)



[1]H NMR Spectrum of PIKU(P)

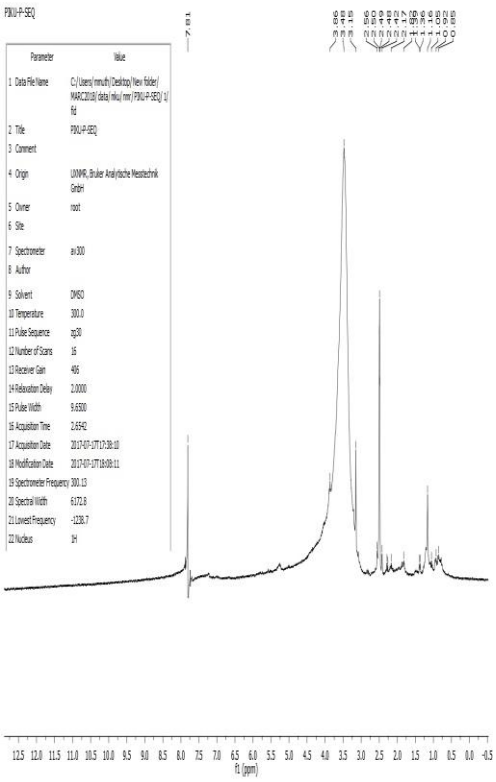


Figure31 [1]H NMR Spectrum of PIKU(P SEQ)

Figure 30

5. SUMMARY AND CONCLUSION

The present study is titled “Phytochemical Profiling of Medicinal Plant *Picrorhiza kurrontha* Be” is focussed on standardisation strategies adopted for validation of medicinal plants and their extracts.

The main objectives of the study are,

- ✓ To carry out the thorough review of literature on *Picrorhiza kurroa*
- ✓ To carry out the phytochemical analysis of extracts of *Picrorhiza kurroa*
- ✓ To determine the total terpenoid content
- ✓ To document the chemical and spectral fingerprinting of extracts of *Picrorhiza kurroa*

Chapter 1 deals with a brief introduction on the significance of medicinal plants in Ayurvedic medicines. Chapter 2 comprises review of literature on the medicinal plant chosen. A thorough search of literature revealed that there are around four hundred reports of various scientific studies carried out with the plant *Picrorhiza kurroa* from 1956 to 2017.

Review of literature is presented in the following section headings,

- Isolation of Chemical Constituents from *Picrorhiza kurroa*
- Quantification of Constituents of *Picrorhiza kurroa*
- Medicinal Potential of *Picrorhiza kurroa*
- Patents on *Picrorhiza kurroa*
- Other Studies on *Picrorhiza kurroa*

Review of literature revealed that the plant the roots and rhizomes of *Picrorhiza kurroa* contain iridoids ad iridoid glycosides mainly. Picrorhizin, Kutkin, picroside I, picroside-II, kutkoside, cucurbitacin, caffeoyl glycosides, caffeic acid derivatives. The quantification of the chemical constituents by robust HPTLC and HPLC methods is also reported. Anumber of patents have been published on herbal compositions containing *Picrorhiza kurroa*.

The methodology adopted in this study is presented in Chapter 3. It includes methodology of extraction procedures, phytochemical screening, phytoconstituent analysis and chromatographic and spectral fingerprinting. Standard methods and protocols are followed.

In Chapter 4 the results obtained are presented along with suitable interpretations. The present study is an attempt to carry out phytochemical profiling of the leaves of the ayurvedic medicinal plant *Picrorhiza kurroa*. The following stages were part of the study.

- Extractive Value
- Oraganoleptic Characteristics of Extracts
- Solubility of Extracts in Common Solvents
- pH of Extracts
- Phytochemical Screening
- Determination of Phytochemical Constituents
- TLC Analysis of Extracts
- Fingerprinting of Extracts

The following is a summary of the outcome of the study.

The extractive value indicates that higher yield of extract concentrates were obtained in the hexane and hydro alcoholic extracts.

The non polar extract was insoluble in water while the polar extracts were soluble in most of the solvents. All polar extracts are slightly basic in nature. From the phytochemical screening tests it was found that the non polar and polar extracts tested positive for terpenoids.The ethyl acetate extract gave a positive yellow color with sodium hydroxide solution indicating flavonoids.The chromatographic and spectral finger prints of the extracts of *Picrorhiza kurroa* are documented in this study.

Picrorhiza kurroa forms part of the herbal mixture of the Jathyadi Ghrita, a potential wound healing agent formulated by Ayurvedic Formulary of India. Scientific validation should be considered while using medicinal plants for making herbal formulations. The present study finds significance since it involves various stages of standardisation of the extracts of the chosen plant and it will form part of the scientific validation of the herbs used in the preparation of the Jathyadi Ghrita.

REFERENCES

Aswal, Bacchan S.; Chander, Ramesh; Chatterji, Sunil K.; Dhawan, Bhola N.; Dwivedi, Yogesh; Garg, Narendra K.; Jain, Poonam; Kapoor, Narinder K.; Kulshreshtha, Dinesh K.; "**Extraction of a hepato protective fraction containing picroside I and kutkoside, from *Picrorhiza kurrooa* is patented"** K.; U.S., 6 pp., Patent, 1992, CODEN: USXXAM

Narayanan Pushpa; Akamanchi K G**" Colorimetric estimation of total iridoid content of *Picrorhiza kurrooa"***Journal of Asian natural products research**,** Volume: 5**,** Issue: 2**,** Pages: 105-11**,** 2003**,**

Bhandari, Pamita; Kumar, Neeraj; Singh, Bikram; Kaul, Vijay K **" Simultaneous determination of sugars and picrosides in Picrorhiza species using ultrasonic extraction and high-performance liquid chromatography with evaporative light scattering detection"**Journal of Chromatography A, Volume: 1194, Issue: 2, Pages: 257-261, 2008,

Jadhao, M. P.; Bhusari, K. P.; Shrikhande, B. K.; Ghormade, J. M.; Shrikhande, V. N **"Development and quantification of HPTLC method for the estimation of kutkin in *Picrorhiza kurroa*"**Research Journal of Pharmacy and Technology, Volume: 2, Issue: 4, Pages: 768-770, 2009

Bhandari, Pamita; Kumar, Neeraj; Singh, Bikram; Ahuja, Paramvir S.**"Online HPLC-DPPH method for antioxidant activity of *Picrorhiza kurroa* Royle ex Benth. and characterization of kutkoside by Ultra-Performance LC-electrospray ionization quadrupole time-of-flight mass spectrometry"**Indian Journal of Experimental Biology, Volume: 48, Issue: 3, Pages: 323-328, 2010

Ashraf, Haleema; Gupta, R. C.; Siddique, Maa; Bhat, M. A.; Akhter, Sabina; Nazki, I. T. **"HPLC-based quantification of picroside-I and picroside-II in Picrorhiza kurroa and its population dynamics in Kashmir Himalaya"**Journal of Medicinal and Aromatic Plant Sciences, Volume: 33, Issue: 4, Pages: 407-410, 2011

Mohammed Rageeb, Mohammed Usman; Yamgar, Surekha U.; Gadgoli, Chhaya H.; Sali, Lalit P.; Tayade, Jayshri A.; Teli, Nilesh B." **HPTLC analysis of rhizomes of *Picrorrhiza kurroa* and Aarogyawardhini bati"** International Journal of Pharmacy and Technology, Volume: 4, Issue: 2, Pages: 4189-4207, 2012

Akhade, Meenakshi S.; Agrawal, Poonam A.; Laddha, K. S **"Development and validation of RP-HPLC method for simultaneous estimation of picroside I,**

plumbagin, and Z-guggulsterone in tablet formulation"Indian Journal of Pharmaceutical Sciences, Volume: 75, Issue: 4, Pages: 476-482, 2013

Rehman, Habib Ur; Ahmad, Jameel; Chester, Karishma; Tajuddin, Tamboli Ennus; Ahmad, Sayeed"**Chemoprofiling and tissue culture studies on *Picrorhiza kurroa* Royle ex Benth. for production of picroside II"**Annals of Phytomedicine, Volume: 3, Issue: 2, Pages: 70-77, 2014

Sah, J. N.; Varshney, V. K.; Dutta, I. C." **HPTLC Assisted Evaluation of Picroside Content in the Accessions of *Picrorhiza kurrooa* Grown at Different Altitudes in Nepal**"Natural Products Journal, Volume: 5, Issue: 2, Pages: 82-90, 2015

Kumar, Dinesh; Kumar, Rakesh; Singh, Bikram; Ahuja, Paramvir Singh" **Reproducible reversed-phase high-performance thin-layer chromatography-based quality-control method for the endangered medicinal plant *Picrorhiza kurroa* Royle ex Benth**"Journal of Planar Chromatography--Modern TLC, Volume: 28, Issue: 3, Pages: 256-261, 2015.

MATERIALS AND METHODOLOGY

RESULTS AND DISCUSSIONS

SUMMARY AND CONCLUSION

REFERENCES

REVIEW OF LITERATURE