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RESEARCH ARTICLES

# Phytochemical profiling using HRLCMS and evaluation of antioxidant and antibacterial activities of Nepalese medicinal plants

**Prashant Neupane**[1] · **Janardan Lamichhane**[1]

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## Abstract

Medicinal plants have been conspicuous source of novel chemicals and bioactive compounds due to illustrious history of use in traditional medicine. Research on Nepalese medicinal plants are still limited to ethnopharmacological studies and qualitative phytochemical screening with a very few studies exploring their biological activities. This study aims to investigate biological activities of these plants and identify bioactive compounds present in each extract. A phytochemical profile of methanolic extracts of selected medicinal plants was established using high resolution (HR)-LCMS. Antioxidant activities were determined using DPPH, ABTS and FRAP assays. Highest DPPH radical scavenging was shown by Padamchal ($IC_{50} = 3.47 \pm 0.09$), ABTS radical were most efficiently quenched by Pashanbed ($IC_{50} = 3.82 \pm 0.63$) and the highest reducing potential was shown by Nirbikhi (FRAP = $61.76 \pm 2.29$ equivalent µg $Fe^{2+}$/ml). The antioxidant activities of Padamchal and Pashanbed was comparable to that of standard Ascorbic acid and Gallic acid. Further, a significant correlation was found between different antioxidant activities and total phenolic/flavonoid contents of each plant extract. Antibacterial properties against five pathogenic microorganisms was established using agar well diffusion and broth microdilution method. The extracts showed considerable inhibition zones ranging from 10–17.5 mm at maximum concentration of 10 mg/ml. Inhibitory effect was observed against *Staphylococcus aureus* at MIC 31.25 µg/ml of Padamchal, against *Escherichia coli* at MIC 125 µg/ml of Ragatsingey, against *Bacillus subtilis* at MIC 250 µg/ml of Nirbikhi, against *Klebsiella pneumoniae* at MIC 250 µg/ml of Ragatsingey and against *Shigella flexneri* at MIC 250 µg/ml of Padamchal. Furthermore, HR-LCMS analysis manifested presence of several compounds of pharmaceutical importance in the plant extracts. These selected medicinal plants contain significant antioxidant and antibacterial activities owing to the presence of prominent bioactive chemicals. The results stipulate a need for further research and bioprospecting of these plants as source of new natural antioxidants and antibacterial agents.

**Keywords** Phytochemicals · Phenolics · Flavonoids · Antioxidant activity · Antibacterial activity · High resolution liquid chromatography-mass spectrometry (HRLCMS) · Medicinal plants

## Introduction

Oxidative stress and antimicrobial resistance are two prominent challenges that demand a significant interest from researchers all around the globe. Oxidative stress is imposed by increased concentration of free radicals particularly reactive oxygen species (ROS) that includes a number of reactive molecules and free radicals derived from oxygen. These molecules, produced as byproducts during the mitochondrial electron transport of aerobic respiration or by oxidoreductase enzymes and metal catalyzed oxidation, have the potential to cause a number of deleterious events (Yoshikawa and Naito 2002). Likewise, Antimicrobial resistance (AR) is the ability of a microorganism to resist the effects of medication that once could successfully treat the microorganism. Irrational use and overuse coupled with evolution and genetic transfer of resistance mechanisms has equipped more pathogenic microorganisms rendering resistance against current antimicrobials. Antimicrobial resistance has been rising with newer resistance mechanisms emerging and spreading globally. This seriously threatens our ability to treat common infectious diseases.

✉ Janardan Lamichhane
ljanardan@ku.edu.np

[1] Department of Biotechnology, Kathmandu University, Dhulikhel, Nepal

Natural products especially plants and microorganisms provide an inexhaustible reservoir of novel molecules that can be developed into new drug. Medicinal plants in particular have been used in traditional medicine since antiquity to maintain holistic health and have provided preventive and curative medicines in infectious conditions. Medicinal plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids, which are known to have immunomodulatory, antioxidant, antimicrobial, antidiabetic and anticancer properties (Savoia 2012).

Plants produce and utilize phytochemicals as natural antioxidants to protect themselves against free radicals and reactive oxygen species. Carotenoids, vitamin C, vitamin E, phenolic acids, flavonoids, tannins, anthocyanins and stilbenes have been widely studied as the primary free radical scavenging and antioxidant compounds in many medicinal plants. These compounds with antioxidant activity often have other useful biological properties related to their ability to scavenge free radicals such as antimicrobial, anti-inflammatory, anti-aging, antihypertensive and anticancer activities (Xu et al. 2017). The additional health benefits of natural antioxidants as opposed to synthetic led to the extraction, isolation of several antioxidant molecules.

Furthermore, phytochemicals are often produced by plants as defense against pathogens. Several phytochemicals extracted from various plants, have shown antibacterial, antifungal and antiviral activity against several human pathogens. Many studies through the years have shown alkaloids, polyphenols, terpenoids and organosulfur compound could play a role in the management of antibiotic resistant bacteria. Alkaloids such as Berberin, Piperine, Reserpine, aaptamine, quinoline, agelasine, chelerythrine, tomatidine and sanguinarine and have been found antibacterial activity (Cushnie et al. 2014). Similarly, plant polyphenols broadly classified as phenolics, stilbenes, catechins or flavonoids confer a wide range of bioactivities including antibiotic activity against resistant pathogens through various mechanisms (Górniak et al. 2019).

Global prevalence of infectious diseases caused by bacteria is a major public health problem. Common human pathogens such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Shigella flexneri, Bacillus subtilis* and *Staphylococcus aureus* have shown resistance against multiple antibacterial drugs. Current study aims to evaluate antibacterial activity five important medicinal plants from high altitude regions of western Nepal against these pathogens. Further, we evaluated the antioxidant activity and correlation between total phenol/flavonoids and antioxidant potential of plant extracts. Furthermore, we attempt to identify important phyto-constituents using chromatographic and mass spectrometric techniques and describe their potential bioactivities.

## Materials and methods

### Sample collection

Plant sample KUPS_5 (*Berginia ciliata*) was collected during September, 2017 from Jaljala forest (location: 28° 27′ 35.6″ N, 82° 43′ 20.3″ E) in Rolpa District of Nepal. Samples of KUPS_1 (*Rheum australe*), KUPS_2 (*Nirbikhi*), KUPS_3 (*Picrorhiza kurroa*) and KUPS_4 (*Ragatsingey*) were collected in October, 2017 from Badimalika region (location: 29°20′52.9"N, 81°28′19.5"E) in Bajura District of Far Western Region of Nepal. Plants were identified by local healers and author using the features illustrated in Medicinal and Aromatic plants of Nepal. Samples were dried at room temperature under the shade and only the dried root part was ground to powder using mechanical grinder and stored in air-tight containers until further use.

### Phytochemical extraction

20 g finely powdered samples were weighed into 500 ml screw-capped reagent bottles and subjected to maceration for 72 h at room temperature using methanol as extraction solvent with occasional shaking. Contents of the bottle were squeezed through a muslin cloth and the filtrate was re-filtered through Whatman filter paper. The solvent was evaporated under reduced pressure to give residues. Dry extracts were suspended in HPLC grade methanol in flat bottom glass tubes and used for all experiments except for antibacterial activity where the extracts were dissolved in DMSO.

### Antibacterial activity

#### Test microorganisms

Standard strains of common pathogenic microorganisms used for antimicrobial study were *Pseudomonas aeruginosa* (ATCC 10145), *E. coli, B. subtilis*, *S. aureus* (ATCC 12600), *K. pneumoniae* (ATCC 13883) and *S. flexneri* (ATCC 12022). The bacterial strains were obtained from the Department of Biotechnology, Kathmandu University and were maintained in Mueller–Hinton agar (MHA) slants at 4 °C.

### Determination of zone of inhibition

Agar well diffusion method was used for qualitative estimation of antimicrobial potential of the plant extracts. Test inoculum of all bacteria was freshly prepared from the stock cultures in Mueller–Hinton broth (MHB). Bacterial cell suspensions were adjusted to 0.5 McFarland turbidity standards

by diluting each inoculum with autoclaved distilled water to prepare $1 \times 10^8$ bacterial/ml inoculum. Mueller–Hinton agar plates were prepared and the inoculum was spread over the entire agar surface using sterile cotton swabs. The plates were then allowed to dry for 5 min after which a hole with a diameter of 6 mm was punched aseptically using a sterile corkborer. 30 µl of plant extract having concentration of 10 mg/ml was introduced into the well. Appropriate standards and control were applied along with the test extracts and the agar plates were incubated overnight at 35 ± 2 °C. The antimicrobial agent in plant extract diffuses in the agar medium and inhibits the growth of the bacteria tested thereby producing an inhibition zone which was measured by using HiAntibiotic Zone Scale (HiMedia).

### Determination of MIC and MBC

Broth microdilution method (Wiegand et al. 2008) with slight modification followed by agar plating method was used to determine MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) of the plant extracts. First of all, each bacterial suspension was adjusted to $1*10^8$ CFU/ml (0.5 McFarland turbidity standards) by diluting overnight culture with autoclaved distilled water. This was diluted further by a factor of 1:100 by adding 200 µl bacterial suspension to 19.8 ml sterile Mueller–Hinton broth. After that, 50 µl of each plant extract was pipetted into the labelled wells of the plate. Then, 100 µl Mueller–Hinton broth was added into each well along with wells for sterility control and growth control. Finally, 50 µl of appropriately diluted bacterial suspension was added into all wells except for the sterility control well, mixed thoroughly and incubated at 35 ± 2 °C for 16–20 h. The plates were observed thoroughly to determine the minimum inhibitory concentration. Further, to determine minimum bactericidal concentration, cultured broth from the wells showing no growth in MIC assay were plated on separate Mueller–Hinton agar plates. The concentration of plant extract in the well that produced no growth on agar plate was considered as minimum bactericidal concentration for the respective species.

### LC/MS profiling and analysis

LC–MS (liquid chromatography–mass spectrometry) analysis of the methanolic extract of all samples was carried out using Agilent (6550 iFunnel Q-TOFs) system consisting of Hip sampler, binary pump, column component, Q-TOF having dual ion source and electrospray ion generation (ESI) with Agilent Jet Stream (AJS). Chromatographic separations were performed using 5 µl of methanolic sample injected with needle wash onto an Agilent UHPLC (Ultra High Performance Liquid Chromatography) system fitted with a Hypersil Gold column (C18 100 × 2.1 mm-3 MICRON). Elution was carried out using solvent A (0.1% Formic acid in Water) and solvent B (90% Acetonitrile + 10% $H_2O$ + 0.1% Formic acid) at a flow rate of 300 µl/min for upto 30 min. For MS experiment, ionization was achieved using Dual AJS ESI system, the capillary voltage was set to 3500 V, gas temperature to 250 °C, the nebulizer pressure to 35 psi and the drying gas flow rate to 13 l/min. Q-TOF data acquisition and mass spectrometric evaluation were carried out using Agilent Mass Hunter software.

### Statistical analysis

All experiments were performed in triplicates. Values for each sample are expressed as the mean ± standard deviation and were subjected to analysis of variance. Statistical analysis was conducted using the Graph Pad Prism Software, Version 8 and SPSS. ANOVA (Analysis of Variance) and Tukeys HSD (honestly significant difference) test was used to determine significant difference in the means. Pearsons correlation coefficient was used to measure linear correlation between tested variables.

## Results and discussions

### Antibacterial activities

The antibacterial activity measured as diameter of zone of inhibition (ZOI) ranged between 10 to 17.5 mm at a maximum concentration of 10 mg/ml for each extract. Figure 1 shows comparison of diameter of the inhibition zones produced by plant extract and standard antibiotic discs. Similarly, MIC ranged from 31.25 to 250 µg/ml against the tested

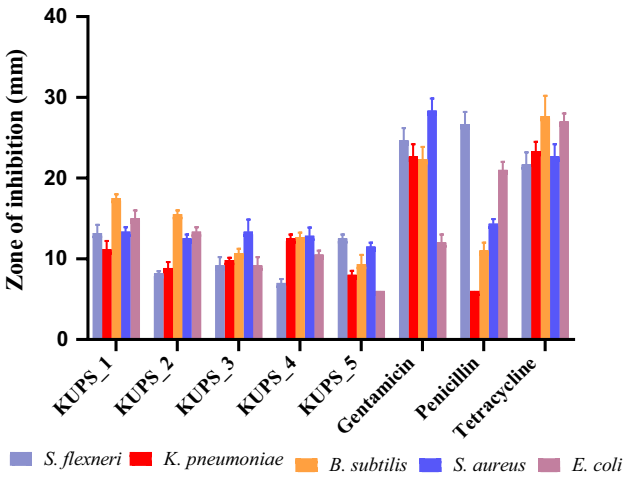


**Fig. 1** Comparision of Zone of inhibition between samples and standards

microorganisms. The ZOI and MIC values of samples and standards are given in Tables 1 and 2 respectively. All plant extracts showed considerable antibacterial effects against the tested pathogens with ZOI > 8 mm except for KUPS_5 (*Pashanbed*) which was inactive against *E. coli*. KUPS_1 (*Padamchal*) produced the most significant antibacterial effect against all tested bacteria.

Methanolic extracts of KUPS_1 (*Padamchal*) was found to inhibit both gram positive (*S. aureus, B. subtilis*) and gram negative (*S. flexneri, K. pneumoniae, E. coli*) bacteria. It was most effective against *B. subtilis* (ZOI 17.5 ± 0.5 mm) and the inhibition was comparable to standard antibiotic Gentamycin (ZOI = 22.33 ± 1.53 mm). Further, methanolic extracts of *Padamchal* produced relatively larger zone of inhibition against *S. aureus* (ZOI 13.33 ± 0.58 mm), *S. flexneri* (ZOI 13.17 ± 1.04 mm) and *E. coli* (ZOI 15 ± 1 mm) compared to other extracts. Considerable inhibition was observed against *K. pneumoniae* with ZOI 11.17 ± 1.04 mm. MIC values of 31.25 µg/ml and 62.5 µg/ml observed against *S. aureus* and *E. coli* respectively correspond to strong antibacterial activity against these pathogens. However, despite producing the highest zone of inhibition against *B. subtilis,* bactericidal concentration could not be determined which suggests a potential bacteriostatic effect against *B. subtilis*. Significant antibacterial activities against wide range of pathogens can be attributed to the presence of bioactive compounds such as Emodin, Chrysophanol, Daidzein, Dihydrogambogic acid and Resorcyclic acid present in the methanolic extracts of KUPS_1. The antibacterial effects of these compounds have been reported in recent studies (Friedman et al. 2003; Hua et al. 2019; Li et al. 2016; Prateeksha et al. 2019).

Antibacterial investigation of KUPS_5 (*Padamchal*) showed moderate inhibition against *S. flexneri* (ZOI 12.5 ± 0.5 mm) and *S. aureus* (ZOI 11.5 ± 0.5 mm). The MIC value for *S. aureus* was found to be > 250 µg/ml. The alcoholic root extracts of *Bergenia ciliata* showed inhibition zones between 6-10 mm against *B. subtilis* and *S. aureus* (Islam et al. 2002; Singh et al. 2016) which is similar to that observed in our study. Similarly, methanolic extracts of KUPS_3 (*Kutki*) produced significant antibacterial effect against both gram positive and negative bacteria with zones ranging from 9 to 14 mm. KUPS_3 was most effective against *S. aureus* with inhibition zone of 13.33 ± 1.53 mm and bactericidal concentration of > 250 µg/ml. The antibacterial activity of methanolic extracts of KUPS_3 was consistent with the findings of (Kumar et al. 2010).

Antibacterial assay of crude extracts of KUPS_2 (*Nirbikhi*) and KUPS_4 (*Ragatsingey*) revealed that both the extracts have potential to inhibit gram-positive and gram-negative bacteria. KUPS_2 was most active against *B. subtilis, E. coli* and *S. aureus* with ZOI 15.5 ± 0.5 mm, 13.33 ± 0.58 mm and 12.5 ± 0.5 mm respectively. It produced ZOI close to 8 mm against *K. pneumoniae* and *S. flexneri.* KUPS_4 on the other hand showed moderate inhibition against *K. pneumoniae, B. subtilis* and *S. aureus* with zone of inhibition values close to 12 mm. Both KUPS_2 and KUPS_4 was effective against *E.*

**Table 1** Antimicrobial susceptibility of selected pathogens against plant extracts and standard antibiotics

| Bacteria | Samples and Zone of inhibition (ø mm) ± standard deviation | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | KUPS_1 | KUPS_2 | KUPS_3 | KUPS_4 | KUPS_5 | GEN10 | P2 | TE30 |
| *Shigella flexneri* | $13.17 \pm 1.04^{b}$ | $8.17 \pm 0.29^{a}$ | $9.17 \pm 1.04^{a}$ | $7 \pm 0.5^{a}$ | $12.5 \pm 0.5^{b}$ | $24.67 \pm 1.53^{cd}$ | $26.67 \pm 1.53^{d}$ | $21.67 \pm 1.53^{c}$ |
| *Klebsiella pneumoniae* | $11.17 \pm 1.04^{cd}$ | $8.83 \pm 0.76^{bc}$ | $9.83 \pm 0.29^{bc}$ | $12.5 \pm 0.5^{d}$ | $8 \pm 0.5^{ab}$ | $22.67 \pm 1.53^{e}$ | $6 \pm 0^{a}$ | $23.33 \pm 1.15^{e}$ |
| *Bacillus subtilis* | $17.5 \pm 0.5^{c}$ | $15.5 \pm 0.5^{bc}$ | $10.67 \pm 0.58^{a}$ | $12.67 \pm 0.58^{ab}$ | $9.33 \pm 1.15^{a}$ | $22.33 \pm 1.53^{d}$ | $11 \pm 1^{a}$ | $27.67 \pm 2.52^{e}$ |
| *Staphylococcus aureus* | $13.33 \pm 0.58^{a}$ | $12.5 \pm 0.5^{a}$ | $13.33 \pm 1.53^{a}$ | $12.83 \pm 1.04^{a}$ | $11.5 \pm 0.5^{a}$ | $28.33 \pm 1.53^{c}$ | $14.33 \pm 0.58^{a}$ | $22.67 \pm 1.53^{b}$ |
| *Escherichia coli* | $15 \pm 1^{d}$ | $13.33 \pm 0.58^{cd}$ | $9.17 \pm 1.04^{b}$ | $10.5 \pm 0.5^{bc}$ | $6 \pm 0^{a}$ | $12 \pm 1^{c}$ | $21 \pm 1^{e}$ | $27 \pm 1^{f}$ |

Different alphabets within a row represent means that are significantly different at $p \leq 0.05$

*GEN 10* Gentamycin 10 mcg disc, *P2* penicillin 2 units disc, *TE30* tetracycline 30 mcg disc

**Table 2** MIC values of plant extracts against selected pathogens

| Bacteria | MIC µg/ml | | | | |
|---|---|---|---|---|---|
| | KUPS_1 | KUPS_2 | KUPS_3 | KUPS_4 | KUPS_5 |
| *Shigella flexneri* | 125 | 500 | 125 | 62.5 | 250 |
| *Klebsiella pneumoniae* | 125 | 500 | 250 | 250 | NA |
| *Bacillus subtilis* | 125 | 125 | 250 | 125 | 500 |
| *Staphylococcus aureus* | 31.25 | 125 | 125 | 625 | 125 |
| *Escherichia coli* | 62.5 | 125 | 125 | 62.5 | 125 |

*coli* with MIC value of less than 125 µg/ml. KUPS_4 also produced bactericidal effect against *K. pneumoniae* and *S. aureus* at concentration of 250 µg/ml.

## LCMS profiling

Phytochemical screening of KUPS_1 (*Padamchal*), through High Resolution (HR)-LCMS detected 13 unknown and 87 known compounds. The LCMS chromatogram (Fig. 2) and high-resolution mass spectrometry analysis showed the presence of compounds like Myricetin, Coumaric acid, Catechin, Catechol, Quercetin, Ferulic acid, Taxifolin, Gallic acid, Dimethyl caffeic acid, Terpenone, Daidzein, Khivorin, Dihydrogambogic acid, Resorcylic acid, Chrysophanol and Emodin among others listed in Table 3. The antioxidant and antibacterial activity of methanolic extract of KUPS_1 may be attributed to a high phenolic and flavonoid content. Plant polyphenols such as Taxifolin, Quercetin, Gallic acid and Resorcylic acid present in the methanolic extract are known antioxidants (Pandey and Rizvi 2009).

Compounds such as epigallocatechin, quercetin, gallic acid, and dimethyl caffeic acid have proven antioxidant properties (Brewer 2011). Similarly, antioxidants like resorcylic acid, taxifolin, catechin, *p*-coumaric acid and myricetin also manifest antibacterial and anti-inflammatory properties (Górniak et al. 2019; Mandal et al. 2017; Semwal et al. 2016). Taxifolin, podophyllotoxin, emodin and chrysophanol have been found to possess multiple pharmacological properties (Guerram et al. 2012; Prateeksha et al. 2019; Su et al. 2005; Sunil and Xu 2019).

Similarly, HR-LCMS analysis of KUPS_5 (*Pashanbed*) revealed the presence of several pharmaceutically important molecules as listed in Table 4. Major known compounds including Epicatechin gallate, Bergenin, Metyrapol, Gallic acid, Aphyllic acid, Catechin, Tetrahydrogambogic acid, Sitosterol and Stigmasterol can be seen as major peaks in the chromatogram (Fig. 3). These compounds have been known to show excellent antioxidant activities in vitro (Bajracharya and Maharjan 2013; Singh et al. 2016). Recent study has indicated that quercetin and catechin can serve as potent antiurolithiasis agents (Sharma et al. 2017). Both these compounds were present in the root extracts of *Berginia ciliata* taken for this study which justifies the ethnomedicinal use of this plant as stone breaker.

Herbal extracts of *Bergenia* are known diuretics and also inhibit the growth and dissolve urinary stones (Saha and Verma 2013). β-Sitosterol was found to improve urinary symptoms and discharge volume and can be useful in treatment of benign prostatic hyperplasia (Rakel 2018). Besides, sitosterol and stigmasterol are known to inhibit cholesterol absorption in intestine thereby reducing levels of cholesterol in blood (Batta et al. 2006; Mattson et al. 1982). Furthermore, Bergenin, which was abundantly found in the root extracts of KUPS_4 (*Berginia ciliata*) is known to have diuretic, antioxidant and antibacterial properties (Singh et al. 2016). Aphyllic acid was reported to have bronchospasmolytic properties along with the ability to inhibit the transmission of impulses from the vagus nerve to the heart, and attenuation of toxic action of anticholinesterase substances (Otargaliev et al. 1976). Apart from these, compounds such as epicatechin gallate and gambogic acid have been found effective against drug resistant bacteria and various cancers (Chu et al. 2017; Pandey et al. 2016; Taylor et al. 2005; Wang and Chen 2012).

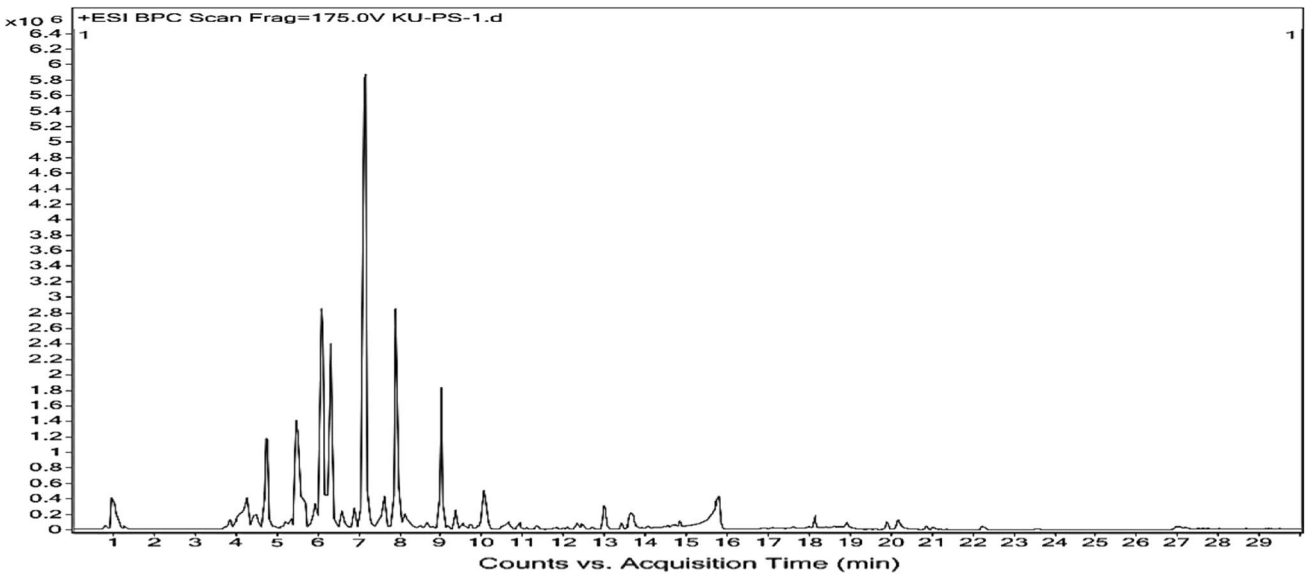


**Fig. 2** LCMS chromatogram of KUPS_1 (Padamchal)

**Table 3** List of compounds identified in the methanolic extract of KUPS_1 (*Padamchal*) by ESI-QTOFMS

| SN | Name | Molecular formula | Retention time | Mass | *m/z* |
|---|---|---|---|---|---|
| 1 | Podophyllotoxin | $C_{22}H_{22}O_8$ | 0.894 | 414.1334 | 431.090 |
| 2 | Myricetin | $C_{15}H_{10}O_8$ | 1.397 | 318.0367 | 319.044 |
| 3 | *p*-Coumaric acid | $C_9H_8O_3$ | 3.341 | 164.0471 | 165.053 |
| 4 | Catechin | $C_{15}H_{14}O_6$ | 3.729 | 290.0786 | 291.085 |
| 5 | Flecainide | $C_{17}H_{20}F_6N_2O_3$ | 3.752 | 414.14 | 397.139 |
| 6 | 3-Hydroxy-DL-kynurenine | $C_{10}H_{12}N_2O_4$ | 5.486 | 224.0784 | 207.075 |
| 7 | Metochlopramide | $C_{14}H_{22}ClN_3O_2$ | 5.697 | 299.1415 | 322.131 |
| 8 | Quercetin | $C_{15}H_{10}O_7$ | 6.126 | 302.0435 | 285.038 |
| 9 | Dimethyl caffeic acid | $C_{11}H_{12}O_4$ | 6.132 | 208.0726 | 191.069 |
| 10 | Nabumetone alcohol | $C_{15}H_{18}O_2$ | 6.467 | 230.1331 | 235.112 |
| 11 | Ferulic acid | $C_{10}H_{10}O_4$ | 6.584 | 194.0526 | 177.054 |
| 12 | Glibornuride | $C_{18}H_{26}N_2O_4S$ | 6.599 | 366.161 | 389.150 |
| 13 | Taxifolin | $C_{15}H_{12}O_7$ | 6.656 | 304.0576 | 287.054 |
| 14 | Methylergonovine | $C_{20}H_{25}N_3O_2$ | 7.573 | 339.1963 | 344.174 |
| 15 | Mitoxantrone dicarboxylic acid | $C_{22}H_{24}N_4O_8$ | 7.129 | 472.1559 | 473.161 |
| 16 | Gallic acid | $C_7H_6O_5$ | 7.907 | 170.0192 | 175.002 |
| 17 | Chrysophanol | $C_{15}H_{10}O_4$ | 8.116 | 254.0556 | 255.062 |
| 18 | 6-Formylindolo[3,2-B] carbazole | $C_{19}H_{12}N_2O$ | 8.637 | 284.0891 | 285.096 |
| 19 | Emodin | $C_{15}H_{10}O_5$ | 10.075 | 270.052 | 271.059 |
| 20 | Methoxyvone | $C_{17}H_{14}O_3$ | 10.087 | 266.0943 | 271.073 |
| 21 | Carteolol | $C_{16}H_{24}N_2O_3$ | 12.971 | 292.1798 | 275.176 |
| 22 | Meloxicam | $C_{14}H_{13}N_3O_4S_2$ | 15.698 | 351.0271 | 356.007 |
| 23 | Resorcylic acid | $C_7H_6O_4$ | 27.123 | 154.0273 | 155.034 |

**Table 4** List of compounds identified in KUPS_5 (*Pashanbed*) by ESI-QTOFMS

| SN | Compound | Molecular formula | Retention time | Mass | *m/z* |
|---|---|---|---|---|---|
| 1 | Epicatechin gallate | $C_{22}H_{18}O_{10}$ | 3.748 | 442.0941 | 465.083 |
| 2 | Bergenin | $C_{14}H_{16}O_9$ | 3.804 | 328.0818 | 311.077 |
| 3 | Metyrapol | $C_{14}H_{16}N_2O$ | 6.244 | 228.1267 | 211.123 |
| 4 | Gallic acid | $C_7H_6O_5$ | 7.081 | 170.0216 | 175.002 |
| 5 | 2E,8E-Undecadiene-4,6-diynoic acid | $C_{11}H_{10}O_2$ | 7.166 | 174.0693 | 175.077 |
| 6 | 8′,10′-Dihydroxydihydroergotamine | $C_{33}H_{39}N_5O_5$ | 9.031 | 585.2944 | 568.291 |
| 7 | 3,7-Epoxycaryophyllan-6-one | $C_{15}H_{24}O_2$ | 9.268 | 236.1829 | 219.179 |
| 8 | Dodecanedioic acid | $C_{12}H_{22}O_4$ | 9.901 | 230.1547 | 213.151 |
| 9 | Dinorpromazine | $C_{15}H_{16}N_2S$ | 10.128 | 256.1036 | 279.092 |
| 10 | Aphyllic acid | $C_{15}H_{26}N_2O_2$ | 10.469 | 266.199 | 249.195 |
| 11 | Metergoline | $C_{25}H_{29}N_3O_2$ | 10.983 | 403.2266 | 404.234 |
| 12 | Catechin | $C_{15}H_{14}O_6$ | 12.743 | 290.0792 | 273.075 |
| 13 | Meloxicam | $C_{14}H_{13}N_3O_4S_2$ | 15.698 | 351.0285 | 356.007 |
| 14 | 3β,5 β Tetrahydronorethindrone disulfate | $C_{20}H_{30}O_8S_2$ | 16.938 | 462.145 | 445.145 |
| 15 | Tetrahydrogambogic acid | $C_{38}H_{48}O_8$ | 17.699 | 632.3483 | 637.326 |
| 16 | 6-Deoxocathasterone | $C_{28}H_{50}O_2$ | 18.941 | 418.3742 | 423.353 |
| 17 | Sitosterol | $C_{29}H_{50}O$ | 20.716 | 414.3867 | 397.383 |
| 18 | Stigmasterol | $C_{29}H_{48}O$ | 27.101 | 412.3711 | 431.378 |

Similarly, Fig. 4 shows the LCMS chromatogram of methanolic extract of KUPS_3 (Kutki). Mass spectrometry showed presence of known compounds such as Picroside II, Picroside III, Pikuroside, 6,7-dimethyl-8-(1-D-ribityl) lumazine, Apocynin, 4-hydroxyquinazoline, entandrophragmin, neoxanthin, 6-deoxotyphasterol and isoreserpine along with 36 unidentified compounds some of which are listed in Table 5. The findings were consistent with previous study

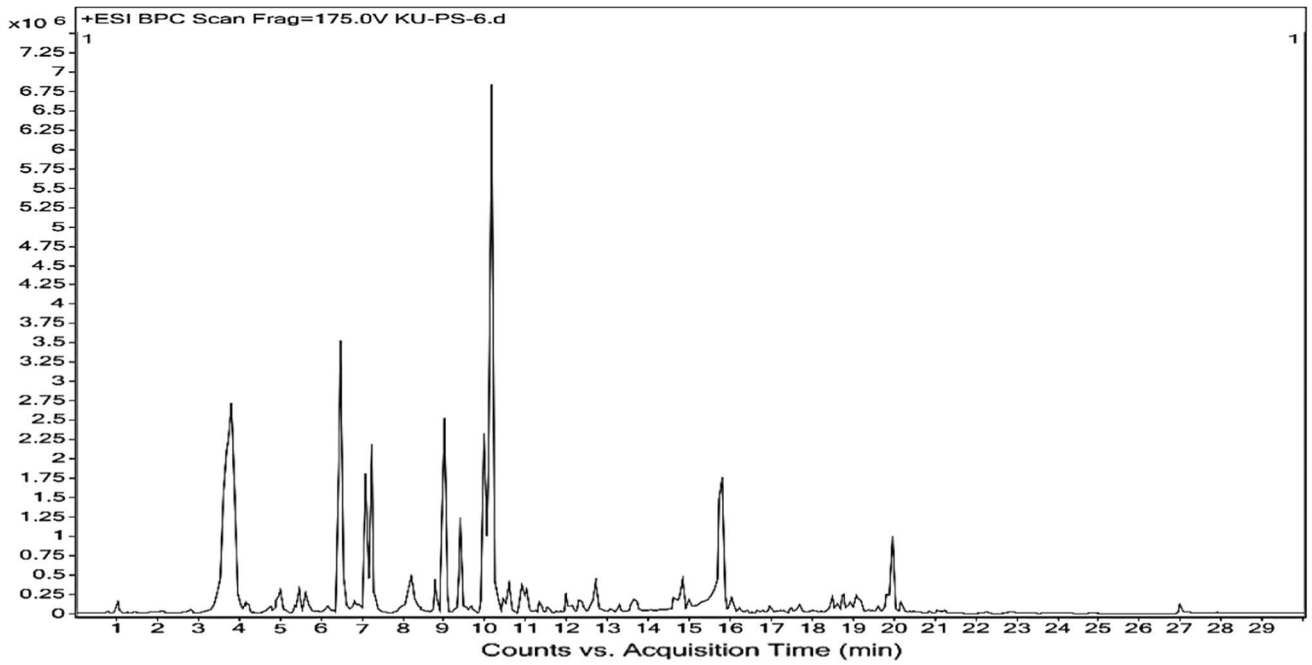


**Fig. 3** LCMS chromatogram of KUPS_5 (Pashanbed)

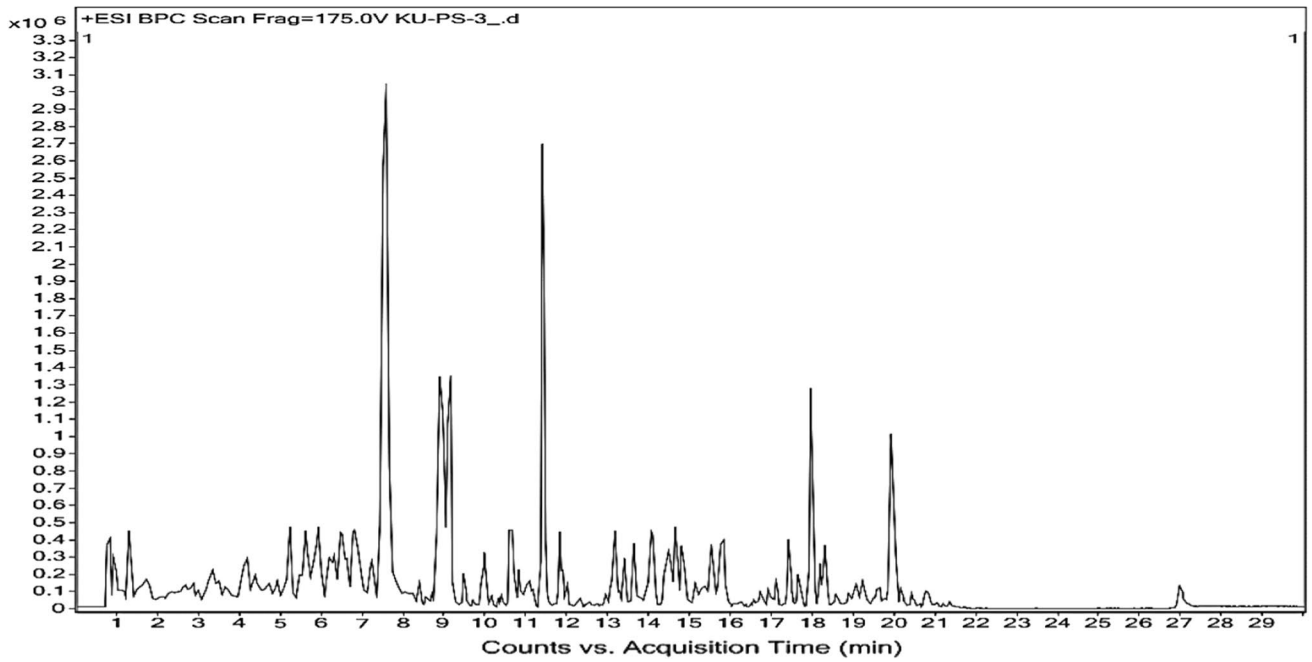


**Fig. 4** LCMS chromatogram of KUPS_3 (Kutki)

by (Masood et al. 2015). Further, Apocynin is known to prevent neutrophil oxidative burst thereby acting powerful antioxidant and anti-inflammatory agent and Picrosides have shown anticancer activities in vitro (Simons et al. 1990; Soni and Grover 2019).

Iridoid glycosides such as Picroside I, II, III and Kutkoside possess various anti-inflammatory, anticancer and hepatoprotective properties (Kumar and Shukla 2017; Soni and Grover 2019). Further picrosides as antioxidants act as neuroprotective agents (Zhai et al. 2017) and also show

**Table 5** List of compounds identified in KUPS_3 (Kutki) by ESI-QTOFMS

| SN | Name | Molecular formula | Retention time | Mass | *m/z* |
|---|---|---|---|---|---|
| 1 | 6-feruloylcatalpol (Picroside III) | $C_{25}H_{30}O_{13}$ | 0.934 | 538.17 | 543.146 |
| 2 | Lyxosylamine | $C_5H_{11}NO_4$ | 1.002 | 149.072 | 150.079 |
| 3 | Desmethylmaprotiline | $C_{19}H_{21}N$ | 4.139 | 263.171 | 264.178 |
| 4 | Kutkoside (Picroside II) | $C_{23}H_{28}O_{13}$ | 4.38 | 512.148 | 513.155 |
| 5 | Pikuroside | $C_{23}H_{30}O_{14}$ | 5.081 | 530.163 | 531.166 |
| 6 | 6,7-Dimethyl-8-(1-D-ribityl)lumazine | $C_{13}H_{18}N4O_6$ | 5.405 | 326.124 | 309.120 |
| 7 | 2-Octenedioic acid | $C_8H_{12}O_4$ | 5.564 | 172.074 | 177.052 |
| 8 | 4,7,10,13,16,19-Docosahexaynoic acid | $C_{22}H_{20}O_2$ | 5.628 | 316.143 | 339.132 |
| 9 | Nalbuphine-3-glucuronide | $C_{27}H_{35}NO_{10}$ | 5.69 | 533.234 | 556.225 |
| 10 | Apocynin | $C_9H_{10}O_3$ | 5.82 | 166.063 | 167.069 |
| 11 | 4-Hydroxyquinazoline | $C_8H_6N_2O$ | 5.989 | 146.049 | 151.027 |
| 12 | Kutkin (Kutkoside) | $C_{23}H_{28}O_{12}$ | 6.135 | 496.158 | 479.147 |
| 13 | Entandrophragmin | $C_{43}H_{56}O_{17}$ | 6.602 | 843.343 | 848.322 |
| 14 | Warfarin alcohol | $C_{19}H_{18}O_4$ | 6.821 | 310.12 | 293.117 |
| 15 | 6,7-Dimethyl-8-(1-Dribityl)lumazine | $C_{13}H_{18}N_4O_6$ | 7.441 | 326.123 | 309.120 |
| 16 | Avermectin A2b | $C_{48}H_{74}O_{15}$ | 8.077 | 890.517 | 895.495 |
| 17 | 1Alpha,25-dihydroxy-2beta-(4-hydroxybutoxy) vitamin D3 | $C_{31}H_{52}O_5$ | 8.927 | 504.37 | 487.367 |
| 18 | 1-Myristoyl-2-oleoyl-sn-glycero-3-phosphate | $C_{35}H_{67}O_8P$ | 9.122 | 646.447 | 651.429 |
| 19 | Ergosterol acetate | $C_{32}H_{50}O_2$ | 9.127 | 464.377 | 487.367 |
| 20 | Neoxanthin | $C_{42}H_{58}O_5$ | 9.524 | 642.417 | 647.399 |
| 21 | Desmethylastemizole | $C_{27}H_{29}FN_4O$ | 11.893 | 444.231 | 445.239 |
| 22 | 6-Deoxotyphasterol | $C_{28}H_{50}O_3$ | 13.225 | 434.369 | 457.358 |
| 23 | Losartan | $C_{22}H_{23}ClN_6O$ | 16.947 | 422.154 | 445.146 |
| 24 | Isoreserpine | $C_{33}H_{40}N_2O_9$ | 17.477 | 608.274 | 609.282 |

promising antidiabetic effects in animal models (Zhu et al. 2016). Similarly, apocynin acts as free radical scavenger and antioxidant in leukocytes and vascular cells (Heumuller et al. 2008). Apocynin was also found to inhibit NADPH-oxidase and effect changes in vascular permeability which can be useful in treatment of inflammatory diseases, arteriosclerosis and hypertension (Anter et al. 2018; Stefanska and Pawliczak 2008). Besides, quinazoline derivatives have been known to have bioactivities such as antibacterial, antifungal, anticonvulsant, anti-inflammatory, anti-HIV, anticancer and analgesic properties (Jafari et al. 2016). Compounds such as phytosterol and reserpine have been widely effective in treatment of arteriosclerosis and hypertension (Cabral and Klein 2017; Gupta et al. 2011; Shamon and Perez 2016).

Similarly, UHPLC chromatogram (Fig. 5) and mass spectrometry analysis of KUPS_2 (*Nirbikhi*) extract establish the presence of active compounds such as Antipyrine, Isoflurophate, Melphalan, Epigallocatechin, Norstictic acid pentaacetate, Ginkgolide C, Convallotoxin, Dipyridamole, Dyphylline and Theaflavin along with a few unsaturated lipids listed in Table 6. Several studies have established strong antioxidant and anticancer activities of compounds such as Epigallocatechin, Theaflavin, Melphalan and Norstictic acid (Iqbal et al. 2017; Leung et al. 2001). Compounds Dipyridamole and Dyphylline have been known for their antidiabetic and vasodilatory effects. Convallotoxin has been found useful in treatment of arrythmias and different cancers in smaller doses. The presence of these high valued active compounds suggests further bioprospecting of KUPS_2 as source of analgesic, anti-inflammatory, antioxidant and anticancer compounds.

*Nirbikhi* is traditionally used as antidote to poisoning related to aconites, mushroom and wild flowers. LCMS revealed the presence of compounds such as dipyridamole, todralazine, isoetharine and dyphylline which are found to be effective as broncho/vasodilator in treatment of bronchitis and asthma (Carvalho et al. 1998; Cohen 1967; Khalil et al. 2005). Ginkgolide C acting as specific platelet-activating factor antagonists can serve as anti-inflammatory and vaso/broncho-dilatory drugs (Papakonstantinou 2018) in addition to their antioxidant and neuroprotective effects. Another compound, Norstictic acid was found to be promising against breast cancer cell line in bioassay guided fractionation assays (Ebrahim et al. 2016).

Furthermore, chromatographic and mass spectrometric analysis showed that methanolic extract of KUPS_4

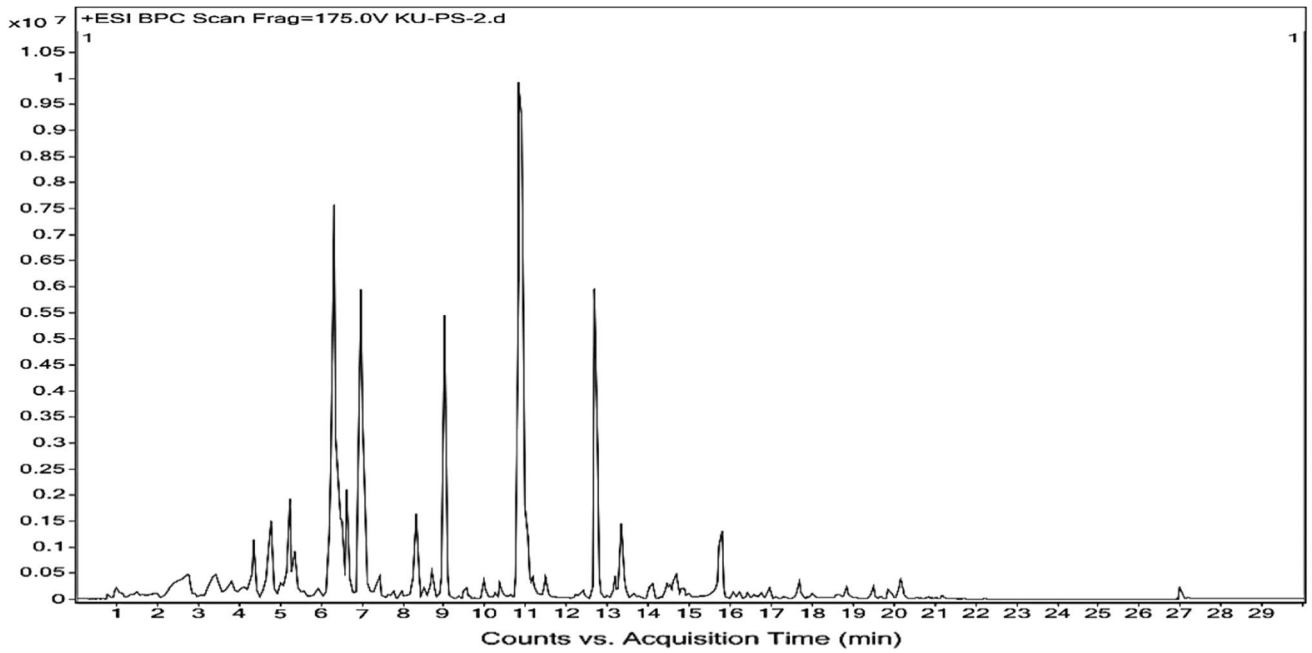


**Fig. 5** LCMS chromatogram of KUPS_2 (Nirbikhi)

**Table 6** List of compounds identified in KUPS_2 (*Nirbikhi*) by ESI-QTOFMS

| SN | Name | Molecular formula | Retention time | Mass | *m/z* |
|---|---|---|---|---|---|
| 1 | 3-*O*-Methylisoetharine | $C_{14}H_{23}NO_3$ | 1.07 | 253.16 | 236.158 |
| 2 | 3-*O*-methyl-L-DOPA | $C_{10}H_{13}NO_4$ | 2.74 | 211.09 | 194.082 |
| 3 | Antipyrine | $C_{11}H_{12}N_2O$ | 3.23 | 188.1 | 171.096 |
| 4 | Todralazine | $C_{11}H_{12}N_4O_2$ | 3.76 | 232.1 | 215.1 |
| 5 | 2E,4E,6E,8E-Decatetraenedioic acid | $C_{10}H_{10}O_4$ | 4.38 | 194.06 | 177.056 |
| 6 | Isoflurophate | $C_6H_{14}FO_3P$ | 4.683 | 184.066 | 167.063 |
| 7 | *N*-Carbamoyl-DL-aspartic acid | $C_5H_8N_2O_5$ | 4.712 | 176.041 | 177.048 |
| 8 | Xanthosine | $C_{10}H_{12}N_4O_6$ | 5.186 | 284.077 | 289.056 |
| 9 | Melphalan | $C_{13}H_{18}Cl_2N_2O_2$ | 5.372 | 304.073 | 287.069 |
| 10 | Epigallocatechin | $C_{15}H_{14}O_7$ | 6.174 | 306.073 | 289.071 |
| 11 | 5-(3,4-Dihydroxyphenyl)-5-phenylhydantoin | $C_{15}H_{12}N_2O_4$ | 6.443 | 284.08 | 289.058 |
| 12 | Zomepirac | $C_{15}H_{14}ClNO_3$ | 6.978 | 291.064 | 274.061 |
| 13 | Glucosamine 6-sulfate | $C_6H_{13}NO_8S$ | 8.347 | 259.036 | 260.043 |
| 14 | Ginkgolide C | $C_{20}H_{24}O_{11}$ | 8.874 | 440.134 | 441.144 |
| 15 | Convallotoxin | $C_{29}H_{42}O_{10}$ | 9.058 | 550.286 | 573.276 |
| 16 | 4,8,11,14-Eicosatetraynoic acid | $C_{20}H_{24}O_2$ | 9.101 | 296.180 | 301.158 |
| 17 | Dipyridamole | $C_{24}H_{40}N_8O_4$ | 10.545 | 504.320 | 509.301 |
| 18 | Norstictic acid pentaacetate | $C_{28}H_{24}O_{15}$ | 10.882 | 600.092 | 601.105 |
| 19 | Dyphylline | $C_{10}H_{14}N_4O_4$ | 11.105 | 254.101 | 259.079 |
| 20 | Testosterone sulfate | $C_{19}H_{28}O_5S$ | 11.264 | 368.166 | 373.145 |
| 21 | Theaflavin | $C_{29}H_{24}O_{12}$ | 12.732 | 564.137 | 569.116 |

(*Ragatsingey*) contains active compounds such as Ethoxyquin, Deguelin, Bicuculline, Ecgonine, Piperidolate, D-erythro-MAPP, Tolazamide, Garcinolic acid, Rosiglitazone as major peaks in chromatogram (Fig. 6) along with other compounds listed in Table 7. Ethoxyquin is considered a highly effective antioxidant molecule (Ramis-Ramos 2003). Tolazamide possesses stimulatory action on β-cells in pancreas and has been used in the treatment of

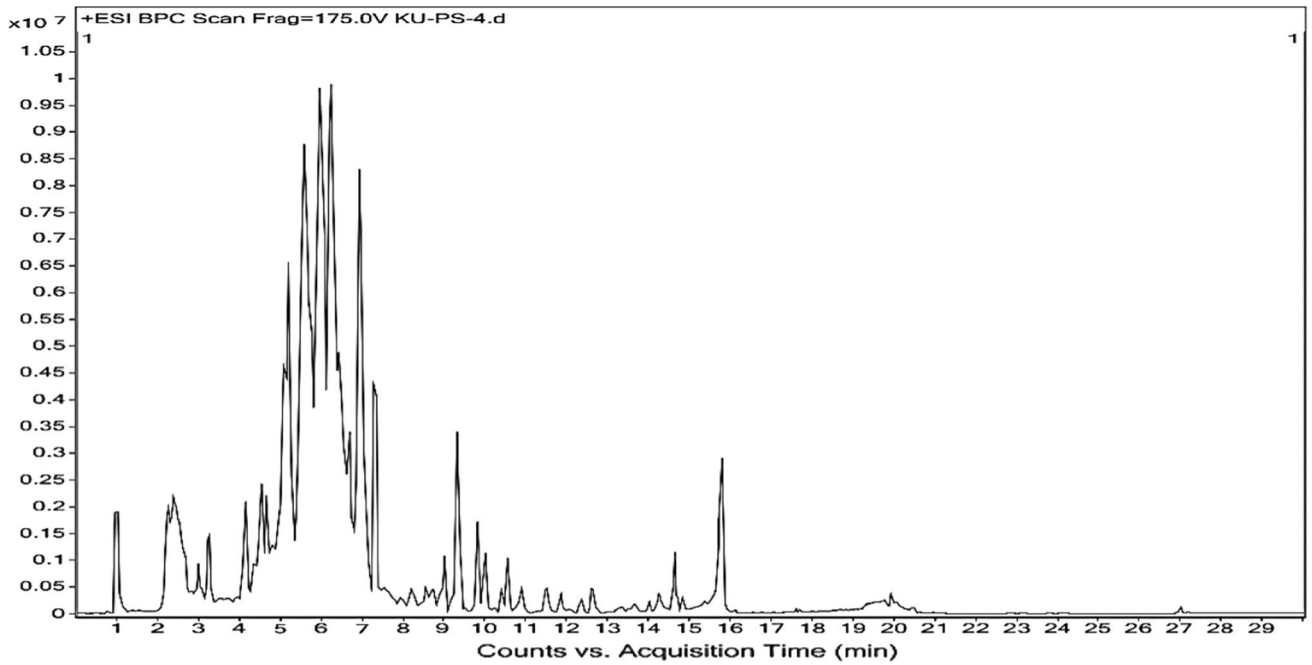


**Fig. 6** LCMS chromatogram of KUPS_4 (Ragatsingey)

**Table 7** List of compounds identified in KUPS_4 (*Ragatsingey*) by ESI-QTOFMS

| SN | Name | Molecular formula | Retention time | Mass | *m/z* |
|---|---|---|---|---|---|
| 1 | Ecgonine | $C_9H_{15}NO_3$ | 2.29 | 185.106 | 190.085 |
| 2 | Normetanephrine | $C_9H_{13}NO_3$ | 2.415 | 183.09 | 188.069 |
| 3 | Ethoxyquin | $C_{14}H_{19}NO$ | 3.248 | 217.147 | 222.126 |
| 4 | Nefopam | $C_{17}H_{19}NO$ | 4.2 | 253.147 | 236.144 |
| 5 | Norprochlorperazine | $C_{19}H_{22}ClN_3S$ | 4.701 | 359.123 | 382.112 |
| 6 | Bicuculline (+) | $C_{20}H_{17}NO_6$ | 5.04 | 367.107 | 368.114 |
| 7 | Piperidolate | $C_{21}H_{25}NO_2$ | 5.107 | 323.187 | 328.165 |
| 8 | *N*-Didesethylquinagolide | $C_{16}H_{25}N_3O_3S$ | 5.182 | 339.163 | 340.171 |
| 9 | Ritodrine sulfate | $C_{17}H_{21}NO_6S$ | 5.755 | 367.108 | 368.115 |
| 10 | D-Erythro-MAPP | $C_{23}H_{29}NO_2$ | 5.814 | 351.218 | 356.197 |
| 11 | Deguelin (−) | $C_{23}H_{22}O_6$ | 6.735 | 394.142 | 395.148 |
| 12 | Garcinolic acid | $C_{38}H_{46}O_9$ | 6.831 | 646.316 | 337.142 |
| 13 | Tolazamide | $C_{14}H_{21}N_3O_3S$ | 6.911 | 311.129 | 334.119 |
| 14 | Desmethylpirenzepine | $C_{18}H_{19}N_5O_2$ | 6.978 | 337.150 | 338.157 |
| 15 | Pergolide sulfone | $C_{19}H_{26}N_2O_2S$ | 7.316 | 346.173 | 351.151 |
| 16 | Salbutamol-4′-*O*-sulfate | $C_{13}H_{21}NO_6S$ | 7.402 | 319.107 | 320.116 |
| 17 | Verteporfin | $C_{41}H_{42}N_4O_8$ | 9.225 | 718.316 | 351.160 |
| 18 | Rosiglitazone | $C_{18}H_{19}N_3O_3S$ | 10.567 | 357.115 | 380.104 |

non-insulin-dependent diabetes mellitus without expressed microvascular complications (Vardanyan and Hruby 2006). Similarly, Deguelin is known to exhibit significant anti-tumorigenesis and anti-proliferative activity in various types of cancers (Wang et al. 2013). Presence of these compounds warrants for further research need on antioxidant, anticancer and antidiabetic properties of KUPS_4.

Chromatography and mass spectrometry analysis of the selected plant samples establishes the abaundance of phenolic compounds. Phenol and flavonoids have been widely studied for their preventive effects against oxidative stress related diseases, several cancers, cardiovascular diseases and neurodegenerative diseases (Bhuyan and Basu 2017). Medicinal plants can serve as sustainable and rich

**Table 8** Total phenol flavonoid content and antioxidant activities of selected plants

| Sample | DPPH $IC_{50}$ (µg/ml) | ABTS $IC_{50}$ (µg/ml) | FRAP Value (µg $Fe^{2+}$/ml) | TPC GAE (µg/mg) | TFC RE (µg/mg) |
|---|---|---|---|---|---|
| KUPS_1 (*Rheum australe*) | $3.47 \pm 0.09^{a}$ | $3.85 \pm 0.16^{a}$ | $61.76 \pm 2.29^{a}$ | $249.58 \pm 7.73^{a}$ | $480.84 \pm 8.81^{a}$ |
| KUPS_2 (*Nirbikhi*) | $26.52 \pm 0.3^{b}$ | $17.05 \pm 0.21^{b}$ | $65.86 \pm 1.54^{a}$ | $98.41 \pm 1.6^{b}$ | $49.95 \pm 7.25^{b}$ |
| KUPS_3 (*Picrorhiza kurroa*) | $30.85 \pm 2.1^{c}$ | $16.96 \pm 1.12^{b}$ | $45.45 \pm 1.1^{b}$ | $59.37 \pm 1.54^{c}$ | $39.08 \pm 2.61^{b}$ |
| KUPS_4 (*Ragatsingey*) | $39.17 \pm 0.96^{d}$ | $15.78 \pm 0.99^{b}$ | $44.41 \pm 2.40^{b}$ | $76.81 \pm 6.6^{d}$ | $50.79 \pm 2.51^{b}$ |
| KUPS_5 (*Berginia ciliata*) | $3.56 \pm 0.1^{a}$ | $3.82 \pm 0.63^{a}$ | $66.23 \pm 1.29^{a}$ | $213.47 \pm 2.1^{e}$ | $188.01 \pm 9.25^{c}$ |
| Ascorbic acid | $3.65 \pm 0.17^{a}$ | NA | $80.67 \pm 2.71^{c}$ | | |
| Gallic acid | NA | $3.99 \pm 0.2^{a}$ | NA | | |

Different alphabets within a column represent means that are significantly different at $p \leq 0.05$

source of phenolic acids, flavonoids, stilbenes, carotenoids and vitamins. These secondary metabolites are excellent reducing agents, free radical scavengers, and quenchers of singlet oxygen and their presence significantly contributes to the antioxidant function of the plant (Miguel 2010). A prior study has established that the antioxdant activities of samples KUPS_1 and KUPS_5 are comparable to that of the standard ascorbic acid and gallic acid as shown in Table 8. Also, significantly higher corrlation was found between the total phenol and flavonoid contents with the antioxidant capacities of these plant extracts (Neupane and Lamichhane 2020).

Evaluation of Antioxidant capacities of methanolic extracts of the selected plants present KUPS_1 (*Rheum australe*) and KUPS_5 (*Berginia ciliata*) as sources of natural antioxidants with activities similar to ascorbic acid and gallic acid. The antioxidant activity strongly correlated with the amount of phenolic and flavonoid content in the plant extracts. However, relatively higher $Fe^{3+}$ reducing ability of sample KUPS_2 (*Nirbikhi*) despite having lower phenol/flavonoid content warrants further exploration for non-phenolic/flavonoid antioxidants in the sample. Furthermore, presence of bioactive compounds with multifaceted properties present these plants as promising candidates for development of new therapeutic agents.

## Conclusion

Present work provides comprehensive evidence to support that these plants have multifaceted properties and validates their use in treatment of various diseases and conditions. Further, all plant extracts produced significant antibacterial activities against both gram-positive and gram-negative pathogenic bacteria emphasizing need for clinical, toxicological and bioavailability studies. However, in-vitro assays cannot truly predict the activity in-vivo and requires further research and testing in biologically relevant conditions. Presence of highly bioactive compounds support in establishing the antibacterial and antioxidant properties. Moreover, significant antibacterial and antioxidant activities and presence of active compounds with promising bioactivities present these plants as potential sources of therapeutic agents and advocates the need for conservation, screening and bioprospecting of more traditional and endemic plants from Nepal.

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## Compliance with ethical standards

**Conflict of interest** Authors declare no conflict of interests.

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