Antioxidant Activity and HR-LCMS Analysis of Phytochemicals Present in the Methanolic Extract of the Rhizomes of *Paris Polyphylla* (Satuwa)

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ABSTRACT

Antioxidant activity and the complete High-Resolution Liquid Chromatography Mass Spectrometry (HR-LCMS) metabolomic profiling of the rhizomes of *Paris Polyphylla* is still unknown. This study mainly focused on the analysis of the phytochemicals present in the methanolic extract of the rhizomes of *P. Polyphylla*. The roots of *Paris polyphylla* is used in traditional medicine as counteragent for snake bite, remedy for insect poison, relieve for wounds, sore throat etc. Like other medicinal plants, root of *Paris polyphylla* contains numerous potential bioactive compounds. Such bioactive compounds have significant antioxidant activity. Quantative measurement of total Flavonoids, phenolics and antoxidants were carried using spectrometric technique based on quercetin, gallic acid and ascorbic acid as the standards. The total flavonoids content is calculated as 38.430.27mg QE/g dry weights. Moreover the total phenolic constitutes 41.470.53 mg GAE/g dw and the total antioxidant comprises 20.690.97 mg Ascorbic acid equivalent/g dw. HR-LCMS analysis conducted in both positive and negative ESI modes revealed the presence of 128 distinct molecules. Root of *P. Polyphylla* possesses valuable antioxidant properties for dietary and medicinal use.

1. INTRODUCTION

The majority of the people in the world use plants and plant products as a curative agent for their disease due to its negligible side effect. The unique property of medicinal plant to cure various diseases is due to the presence of different class of phytochemicals [1]. Phytochemicals are the bioactive molecules which exhibit antioxidant and anti-inflammatory activities. Some biologically active molecules of the plant have anti-carcinogenic property also. The presence of numerous phytochemicals in the medicinal plant is the cause to use plant based product as antimicrobial, antiviral, analgesic, anticancer and antioxidant activity [2, 3]. Various plant extracts are used as traditional medicine. Essential oil obtained from Lemongrass is also used in traditional medicine which showed maximum inhibition of the bacteria S. aureus at concentration of 100 percent [4]. Clinical and epidemiological research focused on fruits, vegetables, grains and other plant parts [5]**.** Phytochemicals are effective in the prevention of cardiovascular disease as well as diabetics. Phytochemicals are also effective in the treatment of bone disease, i.e. Osteoporosis. Gynecological, neurological and immunological disorder can also be addressed using the phytochemicals [6].

Antioxidants present in the plant material are potential for maintaining good health and coronary heart disease in which arteries of heart are unable to supply enough oxygenated blood to the heart. Cancer is also alarming issue in the group of scientists and food manufacturers as consumers are aware about functional food with specific health effect [7]. Antioxidants can inhibit the oxidation of lipids or other molecules by obstructing the chain initiation or propagation reaction [8]. Phenolic acids, phenolic diterpenes and flavonoids have antioxidative effects [9, 10]. Antioxidant plays important role to absorb and neutralize free radicals, decomposes peroxides and quenches singlet and triplet oxygen [11]. A large number of phytochemicals have significant antioxidant magnitude correlated with lower impermanence rates of cancer in human populations [12].

Rhizome decoction of *P. Polyphlla* is used in the treatment of ulcer, diphtheria, epidemic Japanese B encephalitis, appendicitis, lymphadenopathy, tonsillitis, parotitis, mastitis and rheumatism. It alleviates pain and relieves boils, carbuncles, sore throat, and traumatic pain. It is also used for the treatment of the liver, stomach, nose, lungs, throat and breast cancer in Chinese traditional medicine [13]. The potentialities of the phytochemicals for their use to cure various ailments must be evaluated.

Raw plant extract is the composite blend of numerous phytochemicals used to make traditional medicine. Such composite blends of phytochemicals take control to cure particular and complicated disease [14]. The appropriate screening of the phytochemicals is significant to search novel chemical compounds used for the medicine. The extraction, phytochemical screening and High Resolution Liquid Chromatography Mass spectrometry (HR-LCMS) analysis of the rhizomes extract of plant contributed to search appropriate plant species for particular disease control [15].

The preliminary analysis of the extract of rhizome gives key information about total flavonoids, total phenolic and antioxidant content. A small amount of the extract is sufficient for HR-LCMS analysis which reveals the presence of bioactive compounds such as amino acid, fatty acid, triterpenoids, flavonoids, diterpenes, lipid, phenolic compounds, sesquiterpenoids, quinolizidine alkaloid, and benzoquinone. The purpose of this study was to evaluate the rhizomes of *P. Polyphylla* as new potential source of antioxidant and phenolic

compounds. Our study aims to disclose almost all phytochemicals constituents present in the rhizomes of *P. Polyphylla* using HR-LCMS.

2. MATERIALS AND METHODS

The rhizomes of the plant *P. Polyphylla* were collected from its cultivated habitat, Kherbang, Thabang (2300-2400 meter from sea level) of Jaljala area in Thabang Rular Municipality, Rolpa, Nepal. The rhizomes were excavated during flowering season (July-Aug) from 5-10 centimeter below the surface of the soil using spade. Any coarse materials adhered on the roots were removed and first washed with tap water and then with distilled water. The water present on the surface of the sample was blotted dry using blotting papers. The clean and surface dry rhizomes were stored in zipper bags. The samples were carried into the lab for further execution within 3-4 days. It was chopped into small pieces using knife and air dried in shade until it became completely dry. The air dried samples were stored in desiccators at lab temperature to avoid any moisture absorption.

All the reagents/ chemicals used were of analytical grade (Make: Merck/ Qualigens/ CDH). For qualitative analysis, the powdered sample (50g) was packed in Soxhlet extractor and subjected for continuous percolation for 72 h using 500 mL methanol as solvent. The extract was filtered using Whatman filter paper No.1. Then it was evaporated and concentrated on water bath at about 40-45℃ until it became just like curd. The protocol for the extraction was used as in Naik et al. [16]. The methanolic extract was preserved at -20℃ prior further analysis.

For quantitative analysis, 450 g of powdered sample was taken in 1000mL of round bottom flask and soaked in methanol of analytical grade (4 x 500ml) and stirred in every five minutes (about 10-12 hours) for 3-4 days. The extract thus obtained after 72 hours of cold percolation was filtered using Whatman No.1 (0.45µm) filter paper to remove the suspended materials and dried on petri plate under the high speed ceiling fan. The gummy mass of the plant extract was then placed in amber colored glass bottle, sealed using parafilm and stored at -20℃ prior further analysis. The yield of the extract was calculated using the formula:

$$\text{Yield } \% = \frac{Wt.\,of\,crude\,extract}{Wt.\,of\,the\,powdered\,plant\,sample\,taken} \times 100$$

Qualitative phytochemical tests: Preliminary phytochemical analysis of the methanolic extract is carried out following the method used in Naik et al. [16]. The extract was subjected for the tests of the phytochemicals such as alkaloids, flavonoids, steroids, terpenoids, glycosides, phenols, saponins, carbohydrates, and proteins.

Test for alkaloids: The extract is treated with 2N HCl and heated on water bath at 80˚C for 10 minutes. After heating, the solution was filtered and tested.

i. Mayer's test: Few drops of Mayer's reagent is added to part of the above filtered solution and observed for the formation of turbidity or dull white/ yellow cream precipitate.
ii. Wagner's test: Part of the filtrate is treated with the Wagner's reagent and observed for the formation of brown or reddish brown precipitate.

Test for flavonoids: Following tests are carried out for the screening of flavonoids.

i. Lead acetate test: The extract is treated with few drops of 10% lead acetate solution, and then observed for the formation of yellow coloration.
ii. Sulphuric acid test: The extract is used with few drops of conc. H_2SO_4 and observed for its orange color formation.
iii. NaOH test: The extract is stirred with 10% NaOH followed by 5N HCl and observed the color change from orange to yellow.

Test for terpenoids:

i. Salkowski test: 5 mg of extract is treated with 2 ml of chloroform. Then carefully added conc. H_2SO_4 and observed the brown coloured layer at the interference.

Test for saponins: About 5 mg extract is shaken with 5 ml of distilled water and observed for the formation of foam.

Test for carbohydrates: 5 mg of extract is dissolved in 5 ml of distilled water and filtered. The filtrate is used to test the carbohydrate.

i. Molisch's test: The filtrate is agitated with few drops of Molisch's reagent and shaken well. Conc. H_2SO_4 is then added along the side of the test tube and observed for the formation of red or dull violet ring at the interference.

Test for glycosides: 2 ml of the extract, 1 ml of glacial acetic acid and 1 ml of 5 % ferric chloride is added in a test tube and mixed well. Then 3 drops of conc. H_2SO_4 is added to it, shaked and observed for the formation of greenish blue color.

Test for proteins: Few drops of 0.2 % ninhydrin is mixed to 2 ml of the extract and heated to 100˚C temperature, then observed for the formation of blue color.

Test for Steroids:

i. Salkowski test: The methanolic extract of the sample is again subjected for the extraction with chloroform. Thus extracted aliquot is shaken with conc. H_2SO_4 and set aside for some time and observed the formation of red color.

Test for phenols: 1 ml of the extract, 2 ml of distilled water and 5 drops of 10% ferric chloride solution are mixed in test tube and shaken well. The formation of blue or green colour is observed.

Quantitative phytochemical analysis: The quantitative phytochemical analysis is carried out for the estimation of flavonoids and phenols as described in papers Kenneth-Obosi and Khan [17,18].

Determination of total flavonoids: Total flavonoids were determined by using aluminum chloride colorimetric assay method. In this method, quercetin was used as reference standard solution. 0.2000g standard quercetin compound was dissolved in 200 mL of methanol. From this solution, 10 ml was taken and diluted to 100 ml in the same solvent to make 100 µg/mL solutions [19]. Different concentrations (1, 2, 3, 4, 5, 6, 7, 8, 9, 10 µg/mL) of standard solutions were prepared using serial dilution technique. 35µg /mL extract solution was also prepared in the same solvent. Then 10 µl of 10% aluminum chloride solution was added in 50 µl extract solution (10µg/mL) and standard solution (different concentration) separately and incubated at room temperature for 40 minutes. The absorbance of both solutions was measured at 415 nm (maximum absorbance by blank solution) separately using UV-Visible spectrophotometer (UV-1800, Simadzu).

Determination of total phenolic compound: Total phenolic compound was determined by using gallic acid as reference standard solution. In this process, different concentrations of standard solutions of gallic acid were prepared. One mL of Follin-ciocalteu phenol reagent (10%) and 0.5 ml of 7.5% aqueous Na_2CO_3 solution was mixed with 1mL of the root extract (100 µg/mL), and reference solutions separately [20]. Solution thus prepared was then allowed to stand for about 30 min. The absorbance of the solutions was observed at 742 nm (maximum absorbance) separately using UV-Visible spectrophotometer (UV-1800, Simadzu).

Prior to measuring the absorbance of standard and sample solutions, the absorbance of the blank solution was also noted.

Evaluation of antioxidant activity: Evaluation of anti-oxidant activity was carried out using DPPH assay method [17] and Khan et al. [18]. Briefly, ascorbic acid was used as the reference standard. Different concentrations of reference solutions and extract samples solutions (100µg/mL) were prepared. 250 ml stock solution of DPPH (0.04mg/mL) was prepared in methanol. Then 15 ml of stock solution of DPPH was added in 10 ml reference solution and sample solution prepared in methanol and incubated at room temperature for 30 min. Measurement of absorbance was carried out at 515nm (maximum absorbance) using UV-Visible spectrophotometer (UV-1800, Simadzu).

HR-LCMS/MS analysis:

Methanolic extract of rhizomes (i.e. viscous, 500mg) of *P. Polyphylla* was packed in 5ml sample tube made of borosilicate glass and sent to the lab "sophisticated analytical instruments facility (SAIF), IIT, Mumbai India" by TNT courier for HR-LCMS/MS analysis. The HR-LCMS/MS analysis was carried out using (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). Q-TOF data acquisition and mass spectrometric evaluation were carried out using Agilent mass Hunter Software. The optimal running parameters of HR-LCMS, solvent condition and time table are as follows.

Table 1: Acquisition method of HR-LCMS

Parameter	Value
Ion source	Dual AJS ESI
MS Abs. threshold	200
MS/MS Abs. threshold	5
MS Min Rang(m/z)	130
MS max Rang(m/z)	10000
MS Scan Rate (spectra/sec)	1.0
MS/MS Scan Rate (spectra/sec)	1.0
Isolation Width MS/MS	Medium (~4 amu)
Max Precursors Per Cycle	10
Threshold (Abs)	10000
Target (counts/spectrum)	25000.0
Gas Temperature(^{o}C)	250
Gas Flow (l/min)	13
Nebulizer (psig)	35
SheathGas Temp	300
SheathGas Flow	11
Vcap	3500
Nozzle Voltage (V)	10000
Fragmentor	175
Skimmer1	65
Octopole RFPeak	750
Draw speed	100.0 µL/min
Eject speed	100.0 µL/min
Wait time after drawing	2.0 s
Sample flush out factor	5.0
Injection volume	3.0 µL
Wash Time	3.0 s
Flow	0.300mL/min
low pressure Limit	0.00 bar
high pressure Limit	1200.00 bar
Max. flow Ramp up	100.00 mL/min^{2}
Max. flow Ramp Down	100.00 mL/min^{2}
Stop time	30.00 min

Table 2: Solvent composition

	Channel	Ch.1 Solvent	Name 1	Ch2 Solvent	Selected	Used	Percent
1	A	100.0% water V.02	0.1% FA in water	100.0% water V.02	Ch. 2	Yes	95.00 %
2	B	100.0 % Acetonitrile V.02	90% ACN + 10% H_2O + 0.1% FA	100.0 % Acetonitrile V.02	Ch.2	Yes	5.00 %

Table 3: Time table

	Time	A	B	Flow	Pressure
1	1.00 min	95.00 %	5.00 %	0.300 mL/min	1200.00 bar
2	20.00 min	0.00 %	100.0 %	0.300 mL/min	1200.00 bar
3	25.00 min	0.00 %	100.0 %	0.300 mL/min	1200.00 bar
4	26.00 min	95.00 %	5.00 %	0.300 mL/min	1200.00 bar
5	30.00 min	95.00 %	5.00 %	0.300 mL/min	1200.00 bar

Statistical analysis: All experiments were performed in triplicates. Values for each sample are expressed as the mean ± standard deviation.

3. RESULTS AND DISCUSSION

The 450g of the powdered sample on percolation using Soxhlet in methanol for 72 hours yield 67.779g (15.06%) of crude extract. The preliminary phytochemical screening of the crude extract shows the following result.

Table 4: Test results of phytochemical screening of the plant extract

S. No.	Phytochemicals	Result
1	Alkaloids	
	a. Meyer's test	+
	b. Wagner's test	+
2	Flavonoids	
	a. Lead acetate test	+
	b. H_2SO_4 test	+
	c. NaOH test	+
3	Terpenoids	
	a. Salkowski test	+
4	Saponins	+
5	Carbohydrates	
	a. Molisch's test	+
6	Steroids	+
7	Proteins	+
8	Phenols	+

(+) represents the positive result

Quantitative analysis of selected phytochemicals

Flavonoids: Determination of the total flavonoids in the methanolic extract of rhizome is carried out using quercetin as reference standard and different concentrations of reference is prepared for the calibration of the instruments. The regression equation between absorbance and amount of quercetin was Y = 0.0065x (R^2 = 0.996). Similarly, 10µg/mL solution of the sample is also prepared. All samples were observed in UV-Visible spectrophotometer (UV-1800, Simadzu) at the maximum wavelength of 415nm. The TFC is estimated using the formula x (V/m) and expressed as mg QE/g of extract in dry weight. The quantity of flavonoid compounds in solution is found 2.55µg/mL sample solution. The total flavonoids compounds in the dry extract is found 38.430.27mg QE/g.

Total phenolic compounds: Gallic acid is taken as reference standard and the different concentrations of reference standard are prepared for the calibration of the instrument. The regression equation between absorbance and amount of gallic acid was Y = 0.006x+0.106 (R^2 = 0.998). Similarly, different concentrations of the sample solution in µg/mL are also measured in UV-Visible spectrophotometer (UV-1800, Simadzu) at the maximum wavelength of 742nm. The phenolic compound in solution is found to be 27.59 µg/mL sample solution. Moreover the total phenolic compounds in the dry extract constitutes 41.470.53 mg GAE/g.

Antioxidant activity test: Ascorbic acid is taken as reference standard. Different concentrations of reference standard solutions are prepared for the calibration of the instrument. The regression equation between absorbance and amount of ascorbic acid was y = -0.017x + 0.534 (R^2 = 0.989). Similarly, 100µg/mL solutions of the sample were prepared and observed in UV-Visible spectrophotometer (UV-1800, Simadzu) at the maximum wavelength of 515 nm Spectrometer (UV-1800, Simadzu) at the maximum wavelength of 515nm. The amount of antioxidant compound in sample solution is 13.76µg/mL. Furthermore, the total antioxidant compound in the dry comprises 20.69$\pm$0.97 mg ascorbic acid equivalent /g.

Total Phenolic and Flavonoids have been studied extensively because of their preventive effects against oxidative stress related diseases, several cancers, cardiovascular and neurodegenerative diseases [21]. The plenty of total phenolic and flavonoid contents in the extract be possibility of discovering new natural molecules with antioxidants, antibacterial, anticancer and anti-inflammatory properties.

Phenolic acids, flavonoids and other polyphenols are known to

contribute the antioxidant activities in plants [22, 23]. Relatively high flavonoids, phenolics and antioxidant molecules in the extracts suggest that plant phenolics significantly contribute to the free radical scavenging and reducing activity.

HRLCMS Profiling

The methanolic extract of rhizomes is subjected to HR-LCMS analysis. HR-LCMS analysis is conducted in both positive and negative modes which revealed the presence of 128 distinct compounds, out of which 106 compounds are found in the register of molecular library of HR-LCMS, whereas the remaining 22 compounds are not found in the record of molecular library and listed as unidentified molecules.

The compounds are separated using the LC column (ZORBAX EclipseC_{18} (150 × 2.1 mm, 5µm)), and subsequently analysed using mass spectrometer. The chromatograms of the molecules present in the methanolic extract and their Mass spectra capture under positive and negative modes of ionization are illustrated in figure 1 and 2 respectively. The HR-LCMS profile of the molecules along with mass/charge ratio, retention time of chromatogram and mass of the fragmented moiety is mentioned in the table 5 and 6. Some of the identified compounds are listed below.

Table 5: List of some selected identified compounds in HRLCMS profiling

S.N	Name	Molecular Formula	Retention Time (RT)	Mass	Chemical class
1	L-Carnitine	$C_7H_{16}NO_3$	1.008 (+ ESI)	162.1135	Aminoacid derivative
2	BILA 2185BS	$C_{35}H_{46}N_4O_4S$	1.184 (+ESI)	618.3219	aminoacid amide
3	Bruceantinol	$C_{30}H_{38}O_{13}$	3.185 (+ ESI)	606.2315	Quassinoids
4	MET-enkephalin	$C_{27}H_{35}N_5O_7S$	9.041 (+ ESI)	573.229	Opioid peptides
5	Moracin A	$C_{16}H_{14}O_5$	10.187 (+ ESI)	286.0846	2-arylbenzofuran flavonoids
6	Zapotin	$C_{19}H_{18}O_6$	1.039 (- ESI)	342.1126	Flavone
7	Bryostatin 1	$C_{47}H_{68}O_{17}$	9.987 (- ESI)	904.4498	cyclic macrolides
8	Dioscin	$C_{45}H_{72}O_{16}$	12.672 (- ESI)	868.4759	phytosteroid sapogenin

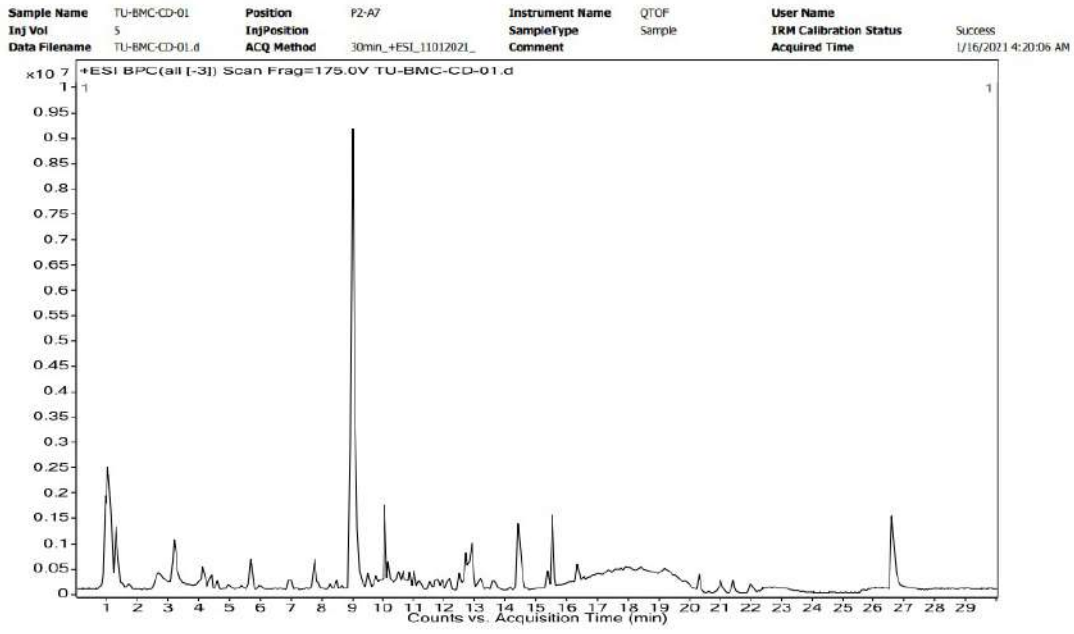

Fig. 1: LCMS chromatogram of the methanolic extract in positive ESI mode

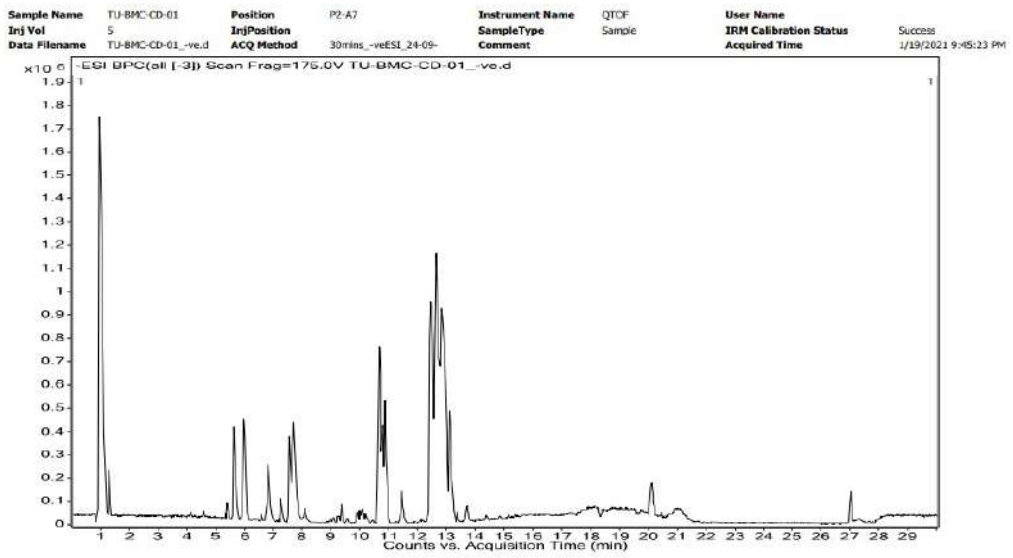

Fig. 2: LCMS chromatogram of the methanolic extract in negative ESI mode.

LCMS spectra, structure & functions of the selected phyto-compounds present in the plant.

To the best of our knowledge, this is the first validate analytical report of *P. Polyphylla* from Rolpa, Nepal. The most intense peak is obtained at retention time (RT) of 9-minute in positive ESI mode and 1min and 13 minute RT in negative ESI mode. Mass to charge ratio (m/z) of the majority of phyto-constituents lies in between100- 1000 mDa range. Out of 106 compounds that are matched in the molecular library of HR-LCMS, significant secondary metabolites are listed in the table 6. Most active secondary metabolites present in the methanolic extract are: L-Carnitine (Organic acid, m/z = 144.1024), BILA 2185BS (Peptide, m/z = 657.2856), Bruceantinol (Degraded triterpenoids, m/z = 321.1499), MET-enkephalin (Peptide, m/z = 573.2517), Moracin A (Phenylpropanoids, m/z = 286.1078), Zapotin (Polyphenols, m/z = 341.1053), Bryostatin 1 (Avermectins m/z = 949.4502/903.4449 /903.4464), Dioscin (Saponin, m/z = 913.4743) and so on.

Table 6: HR-LCMS data sheet of the chemical constituents present in the crude extract

N.	RT Min.	Mass	Ion Type	m/z	Calc m/z	Molecular Formula	Proposed Metabolites	Class of Compounds	Activity
ESI (+)									
1.	1.008	162.1135	M+ [H2O]	144.1024	144.1019	$C_7H_{16}NO_3$	L-Carnitine	Amino acid	Metabolomic Enhancer
2.	1.114	295.1595	(M+ Na) + [-H2O]	300.1383	300.1393	$C_{16}H_{25}NO_2S$	Tertatolol	Sulfonyl	Blood Pressure Depressor
3.	1.116 /1.446	217.1846 217.185	(M+K) +	256.1481 /256.1483	256.1462 /256.1462	$C_{15}H_{23}N$	Prolintane	Alkylamine	Stimulant
4.	1.184	618.3219	(M+K) +	657.2856	657.2871	$C_{35}H_{46}N_4O_4S$	BILA 2185BS	Peptide	Anti- HIV
5.	1.505	142.0204	(M+NH_4) +	160.0542	160.0539	$C_5H_6N_2OS$	Methylthiouracil	Thiazolidinone	Antithyroid reagent
6.	1.885	204.1171	(M+K) + [-H2O]	225.0697	225.0676	$C_{13}H_{16}O_2$	Isobutyl cinnamate	Aromatic Ester	Flavorant
7.	2.79	304.1109	(M+H) + [-H2O]	287.1073	287.109	$C_{11}H_{21}N_4O_2PS$	Imicyafos	Organophosphate	Nematicides
8.	3.185	606.2315 /606.2318	(M+2 (NH4))+2	321.1499 /321.1499	321.1494 /321.1494	$C_{30}H_{38}O_{13}$	Bruceantinol	Quassinoid	Antitumor activity
9.	3.856 /4.18	541.1302 /541.1302	(M+NH4) + [-H2O]	541.1534 /541.1534	541.1557 /541.1557	$C_{26}H_{21}F_6NO_5$	Acrinathrin	Fluoroquinolone	Insecticidal activity
10.	4.426	342.1472	(M+NH4) + [-H2O]	342.1706	342.17	$C_{20}H_{22}O_5$	Deoxymiroestrol	Steroidal phenol	Estrogenic
11.	5.723	480.3094	(M+H) +	481.3167	481.316	$C_{27}H_{44}O_7$	26-Hydroxy ecdysone	Steroids	Ecdysone
12.	9.01 /9.321	336.1047 /336.1053	(M+H) + [-H2O]	319.1014 /319.1022	319.0999 /319.0999	$C_{17}H_{20}O_5S$	Firocoxib	Coxibs	Anti-inflammatory drugs
13.	9.041	573.229	(M+NH4) +[-H2O]	573.2517	573.249	$C_{27}H_{35}N_5O_7S$	MET-enkephalin	Opioid peptide	Anti- cancer / Multiple

									Biological activity
14.	9.932	928.5045	(M+H)+ [-H2O]	911.5014	911.4999	$C_{47}H_{76}O_{18}$	Hoduloside IV	Triterpenoid saponin	Nutraceutical, Anti-sweet
15.	10.187	286.0846	(M+NH4)+ [-H2O]	286.1078	286.1074	$C_{16}H_{14}O_5$	Moracin A	Stilbenoid	Antioxidant &Multiple Biological Activity
16.	10.76 /11.071	430.3089 /430.3086	(M+H)+ [-H2O]	413.3058 /413.3058	413.305 /413.305	$C_{27}H_{42}O_4$	Schidigeragenin C	lignan glycosides	Nutraceutical
17.	11.6	460.3406	(M+NH4)+	478.3745	478.368	$C_{32}H_{44}O_2$	All-trans-carophyll yellow	Triterpenoids	Adjuvant, biomarker
18.	11.817	304.2775	(M+NH_4)+[-H2O]	304.3008	304.2999	$C_{21}H_{36}O$	3-Pentadecylpheno	Phenolic lipids	Multibiological
19.	12.435 /12.716	432.3246 /432.3243	(M+H) + [-H2O]	415.3214 /415.321	415.3207 /415.3207	$C_{27}H_{44}O_4$	24-Hydroxycalcitriol	Secosteroid	Osteoprotective
20.	12.919	872.4781	(M+Na)+ [-H2O]	877.4569	877.4556	$C_{44}H_{72}O_{17}$	Schidigerasaponin D1	Triterpenoid saponin	Antifungal
21.	13.566	326.3795	M+	326.379	326.3781	$C_{22}H_{48}N$	Didecyldimethylammonium	Quaternaryammonium compound (QAC)	Disinfectant
22.	14.437	520.3408	M+	520.3402	520.3398	$C_{26}H_{51}NO_7P$	1-Linoleoylglycerophosphocholin	lysophospholipid	Diagnostic
23.	15.335	358.9574	(M+NH4)+ [-H2O]	358.9805	358.9801	$C_9H_{11}Cl_2N_3O_4S_2$	Methyclothiazide	Thiazide	Diuretic
24.	18.898 /26.653	301.299 /273.2676	(M+H)+	284.2957 /274.2749	284.2948 /274.2741	$C_{16}H_{35}NO_2$	Sphinganine	Sphingolipid	Biomarker Property
25.	20.979	960.5798	(M+H)+	961.5873	961.5843	$C_{48}H_{84}N_2O_{17}$	Megalomicin C1	Macrolide	Antibiotic
26.	26.651	212.1065	(M+NH4)+	230.1403	230.14	$C_{12}H_{12}N_4$	MeIQ	Heterocyclic amine	Carcinogen
27.	26.763	241.132	(M+H)+ [-H2O]	224.1287	224.1281	$C_{12}H_{19}NO_4$	N-(3-oxo-ctanoyl)-homoserine lactone	N-acylhomoserinelactone (AHL)	Priming
ESI (-)									
28.	0.853	361.11	(M-H)-	360.1031	360.1024	$C_{17}H_{19}N_3O_4S$	Omeprazole sulfone	sulfoxides	reduce gastric acid
29.	1.031 /1.378	378.0889 / 378.0885	(M-H)-	377.0822 / 377.0817	377.0813 /377.0813	$C_{17}H_{18}N_2O_6S$	Carbenicillin	Penicillins	Bactericidal antibiotic
30.	1.039	342.1126	(M-H)-	341.1053	341.1031	$C_{19}H_{18}O_6$	Zapotin	Methoxylated flavone	Antidepressant, Anticancer, Antifungal, and Antioxidant agent.
31.	1.24	422.0782	(M+CH3COO)-	481.0913	481.0964	$C_{12}H_{23}O_{14}P$	Lactose 6-phosphate	Disaccharides	Emulsifying salt
32.	4.038	508.1298	(M+HCOO)-	553.1277	553.1231	$C_{23}H_{25}ClN_2O_9$	Clomocycline	Tetracyclines	Tetracycline antibiotic
33.	6.994	1082.4664	(M-H)-	1082.4664	1081.4497	$C_{52}H_{74}O_{24}$	Mithramycin DK	polyketides	Anti-cancer
34.	7.543	340.1749	(M+HCOO)-	385.1751	385.1769	$C_{20}H_{24}N_2O_3$	3-Hydroxyquinine	Quinoline alkaloids	12 % antimalarial
35.	9.987 /12.03 /12.847	904.4498 /904.449 /904.4514	(M+HCOO)- /(M-H)- /(M-H)-	949.4502 /903.4449 /903.4464	949.4439 /903.4384 /903.4384	$C_{47}H_{68}O_{17}$	Bryostatin 1	polyketides. macrolides	Antineoplastic / Neuroprotective
36.	10.628	436.0388	(M+HCOO)-	481.0368	481.0381	$C_{18}H_{16}N_2O_7S_2$	C.I. 14700	Xanthene dyes.	cosmetic colorant
37.	10.843	916.4596	(M-H)-	915.4524	915.4595	$C_{45}H_{72}O_{19}$	Fistuloside C	Triterpenoid Saponins	Anti-fungal
38.	12.672	868.4759	(M+HCOO)-	913.4743	913.4802	$C_{45}H_{72}O_{16}$	Dioscin	Steroidal saponins	Anti-fungal
39.	13.35	668.3472	(M+HCOO)-	713.3468	713.3449	$C_{25}H_{44}N_{14}O_8$	Capreomycin	Cyclic polypeptide	Antibiotics
40.	13.539	381.9669	(M+HCOO)-	426.9652	426.9636	$C_{14}H_8Cl_3F3N_2O$	Fluopicolide	Pyridyl-ethyl-oxazolidinones	Fungicide
41.	19.063	946.569	(M+HCOO)-	991.5697	991.5483	$C_{48}H_{82}O_{18}$	Ginsenoside Re	Ginsenosides Triterpenoid saponins	Anti-arrhythmic
42.	20.082 /20.347	564.3877 /564.3885	(M+HCOO)-	609.388 /609.388	609.3949609.3949/	$C_{40}H_{52}O_2$	Alloxanthin	Xanthophylls carotenoids	Antioxidant

L-Carnitine: It is an important amino acid. It plays a significant role to boost our body metabolism. It does this by improving mitochondrial function and increasing cellular energy. It is also used for fat loss. Besides, L-carnitine supplementation can also help with cognition and better brain functioning and its supplementation may slow down the aging process due to its effect on cellular health. It also helps in some other functions in body, such as maintaining general brain function and reduces certain risk of disorder [24].

BILA 2185BS: It is used as protease inhibitor, i.e., antiviral drug used against HIV. It interrupts HIV replication by binding and blocking HIV protease. For this purpose, the substrate analog protease inhibitor BILA 1906BS and BILA 2185BS are selected combinely [25].

Bruceantinol: It is the novel therapeutic STAT3 (Transducer and Activator of Transcription-3) inhibitor demonstrating potent antitumor activity in-vitro and in-vivo human colorectal cancer models. It also behaves as cytotoxic agent on breast cancer cells too. It effects on the growth of cell, proliferation, cell cycle, and apoptosis. It can be used either alone or in signal transducer and activator of transcriptions-3 gene which is generally associated with disease [26].

MET-enkephalin: It is synthetic form of the naturally occurring, endogenous opioid peptide, it has potential analgesic, neuromodulatory, immunomodulatory, anti-inflammatory, anti-nociceptive/analgestic, antidepressant, and anti-gastrointestinal (GI) motility modulating activity. It also mimics its endogenous ligand and targets, binds and activates the opioid receptors. These leads to an analgestic effect, inhibits neuropathic pain, and inhibits gastrointestinal (GI) muscles contractility, enhances the tissue growth and regeneration, modulates the inflammatory immune response and inhibits the secretion of pro-inflammatory cytokinines. MET-enkephalin with tridecactide is on clinical trial to evaluate the efficiency and safety of an immunomodulatory therapy for the treatment of patients with moderate to severe COVID-19 infections [27].

Moracin A: The therapeutic effect of moracins as 2-arylbenzofuran derivatives against airway inflammation. It is also cytotoxic against THP1 cell. Due to presence of benzofuranheterocycle, these are biologically active as anticancer, antimicobrial, immunomodulatory, antioxidant, and anti-inflammatory property [16].

Zapotin: The study reports the potent anticancer activity of zapotin and suggests a role for zapotin both as a chemopreventive and a chemotherapeutic agent against colon cancer [28].

Bryostatin 1: Bryostatins are potent agonists (drugs or naturally occurring substances that activate physiologic receptors) of protein kinase C. Bryostatin's anticancer activity has been proved against various cancer types [29].

Dioscin: Dioscin is a typical saponin with multiple pharmacological activities. It has demonstrated antitumor activity against many kinds of tumors such as lung cancer, esophageal cancer, gastric cancer, colon cancer, gliblastoma, cervix carcinoma, ovarian cancer, breast cancer, prostate cancer, and leukemia [30].

HR-LCMS screening indicates that root extract of *P. Polyphylla* possesses different bioactive ingredients having multi-variant activates, i.e., useful to suppress the several oxidative stress related diseases. Data shows that crude extract is enriched with significant amount of potential chemical compounds such as phenolics, flavonoids and terpenoids that exhibits correlation with numerous medicinal effects.

Phytochemical screening of the extract showed that it is rich of phytochemicals. It showed positive tests for alkaloids, flavonoids, terpenoids, proteins, phenols, saponin and steroids and carbohydrates.

Quantitative analysis of the selected phytochemicals (flavonoids, phenols and anti-oxidant molecules) suggests that the plant contains substantial amount of those analyzed photochemical. Results showed that total flavonoids compounds (TFC) is 38.43 ± 0.27 mg QE/g, total phenolic compounds (TPC) is 41.810.72 mg GAE/g and anti-oxidant active compounds is 25.750.50 mg Ascorbic acid (AA)/g concentration.

HRLCMS results showed that the plant contain various active phyto-compounds that are very useful for human. The compounds present in the plant are protease inhibitor, antiviral, anti-thyroid, antimicrobial, nematocide, STAT3 inhibitor, insecticide, analgesic, neuro-transmodulator, immunomodulatory, anti-inflammatory, analgesic, antidepressant, membrane stabilizer, cyto-toxic against TPH1 cell, anticancer or antioxidant, antifungal, antibiotic, anti-parasitic, etc. and some compounds are very useful for edema, cirrhosis, kidney disease, control of high blood pressure, boosting body metabolism, loosing fat, brain functioning, insect moulting, controlling osteoarthritis, stimulating intestinal calcium transport, urinary tract infection etc.

Plant extract not only contains beneficial molecules for human health but also contains some molecules which show hazardous effect to human health. They cause insomnia, nervousness, hallucination, psychosis, and even may cause death. Some are acute toxic to oral, dermal, eye and carcinogenic. The molecule Carbenicillin ($C_{17}H_{18}N_2O_6S$) is present in the HRLCMS profile of the extract. It is a penicillin antibiotic. This is only used to treat certain kinds of bacterial infections. Besides its use and benefits, some side effects might be seen while using this medicine. Some side effects are allergic reactions, breathing problems, discolored tongue, fever, pain or difficulty passing urine, stomach cramps, unusual bleeding, bruising, unusually weak or tired, diarrhea, headache and loss of appetite, nausea, vomiting, sore mouth or tongue, stomach upse. Because of its medicinal property MET-enkephalin with tridecactide is on clinical trial to evaluate the efficiency and safety of an immunomodulatory therapy for the treatment of patients with moderate to severe COVID-19 infections [27].

4. CONCLUSION

Methanolic extract of the rhizomes of the plant *P. Polyphylla* showed that the plant is very useful because its quantitative results showed promising amounts of total flavonoids compounds, total phenolic compounds and antioxidant active compounds. It is also rich in different phytochemical compounds like alkaloids, steroids, etc.

HRLCMS profiling shows 128 different compounds (22 unknown compounds and 106 known compounds). Among them, 40 compounds were biologically active and potent and out of 40, 36 of them have useful medicinal properties. They are very useful for treatment of different types of diseases and infections caused by gram positive and gram negative bacteria, virus, and fungus. They are also helpful in treatment of different types of cancers like in-vitro and in-vivo human colorectal cancer, advanced pancreatic cancer, advanced head and neck cancer, lung cancer, esophageal cancer, gastric cancer, colon cancer, gliblastoma, cervix carcinoma, ovarian cancer, breast cancer, prostate cancer, leukemia etc. They are potent in treatment of high blood pressure too.

Among their benefits, the plant has some phyto-compounds like MeIQ, Prolintane, 3-Pentadecylphenol, Didecyldimethylammonium, etc. These compounds cause adverse effects on human health. These are carcinogenic, cause insomnia, nervousness and irritability. Over dosage of them may cause hallucinations, psychosis, and death. They have acute toxicity in oral ingestion too. These compounds not only cause skin and eye irritation. But also have effect on body weight, blood, bronchoalveolar lavage (BAL), and the lungs.

To sum up *P. Polyphylla* is a very potent medicinal plant. Its natural flora (indigenous native plant) is declining alarmingly. Government should initiate a campaign program to cultivate, protect and conserve its natural species. To disclose all the parameters of the root extract of the *P. Polyphylla,* further more analysis of bioactivity in different bacterial strain, isolation, purification and characterization of bioactive compound should be carried out. The unidentified molecules observed in HRLCMS data should be isolated and identified to add further medicinal importance of the plant species. HR-LCMS metabolomic analysis disclosed almost all the active molecules which are proposed to be purified in the future work & used as potential biomarkers.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing interests or personal relationships that could have appeared to influence the work reported in this paper.

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