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Research Article

Phytochemical Profiling of *Passiflora Quadrangularis* Leaves Via LCMS: A Study of Bioactive Compounds

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ABSTRACT
Chemical entities are becoming increasingly interested in medicinal plants and herbal preparations because of their dependable pharmacological activities and accessibility to the general public, which makes them useful in the treatment of a wide range of illnesses. The plant *Passiflora quadrangularis* (Passifloracea), also referred to as "Akashavellari" locally, is utilized in many traditional medicines. This study aims to evaluate the chemical components of Passiflora quadrangularis leaf extracts using Liquid Chromatography-Mass Spectrometry (LCMS). The dried leaves have been extracted with a hydroalcoholic solvent (water: ethanol, 1:1) using Soxhlet extraction. The LC-MS study using the 6540ba Qtof - Infinity 1290 model from Agilent Technologies. It employed LC-ESI/MS in both positive (M + H) and negative (M − H) ionization modes. The separation process employed a C18 reversed-phase column at a temperature of 40 °C. The eluent used was a mixture of acetonitrile and methanol, along with 0.1% formic acid. The analysis revealed the presence of alkaloids, phenolic compounds, amino acids, dicarboxylic acids, flavonoids, and glycosides. Among them, Retronecine was found to be the most prevalent. The negative mode largely accentuated the presence of flavonoid and isoflavonoid glycosides, polyphenols, fatty acid derivatives, and phenolic compounds. Among them, Quercetin 3,7-dirhamnoside was found to be the most abundant. The presence of Kaempferol 3-rhamnoside 7-xyloside was identified in both modalities. The wide range of discovered chemicals highlights the considerable pharmacological potential of Passiflora quadrangularis extracts for therapeutic uses.

Keywords: *Passiflora quadrangularis,* hydroalcoholic extraction, LC-MS, Retronecine, Quercetin 3,7-dirhamnoside

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INTRODUCTION

Phytochemical analysis involves both qualitative and quantitative analysis. While qualitative analysis is concerned with the presence or absence of a phytochemical, quantitative analysis accounts for the quantity or the concentration of the phytochemical present in the plant sample.

Plant metabolism assigns phytochemicals to one of two categories: main or secondary metabolites. Plants require primary metabolites, which include lipids, proteins, amino acids, carbohydrates, purines, and pyrimidines of nucleic acids. Conversely, the remaining plant compounds produced by the cells via metabolic pathways that are derivable from the core metabolic pathways are known as secondary metabolites. These chemical constituents, which are thought to be antibiotic, antifungal, and antiviral, shield plants from infections. They are also essential UV-absorbing chemical components that shield leaves from serious light-induced damage. Plant secondary metabolites have been used for ages in traditional medicine due to their significant biological activity; these molecules are responsible for the therapeutic benefits of the plants. *Passiflora quadrangularis* is an evergreen climbing shrub, attaching itself to other plants by means of coiling tendrils. A very vigorous species, it can normally produce stems up to 15 meters long, but plants in some areas, particularly Java, have been much more

vigorous with some producing stems up to 45 meters long. The edible fruits are greatly appreciated and are often gathered from the wild. The plant is also widely cultivated in many areas of the tropics, especially S. America, both for its edible fruit and also as an ornamental plant.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

Leaves of the plant were collected from local areas of Thrissur, Kerala. The plant materials were taxonomically identified by senior scientist, Dr V B Sreekumar, Forest Botany Department, KFRI, Peechi, Thrissur.

EXTRACTION OF PLANT USING HYDRO-ALCOHOLIC SOLVENT

The materials were shade dried and coarsely powdered. The powdered material extracted with hydro-alcoholic solvent (water: ethanol, 50:50) by using Soxhlet apparatus.

PHYTOCHEMICAL SCREENING OF LEAF EXTRACT

Test for tannins (Ferric chloride test). Two millilitres (2 mL) of the aqueous solution of the extract were added to a few drops of 10% Ferric chloride solution (light yellow). The occurrence of blackish blue colour showed the presence of gallic tannins and a green-blackish colour indicated presence of catechol tannins.

Test for saponins (Frothing Test). Three millilitres (3 mL) of the aqueous solution of the extract were mixed with 10 mL of distilled water in a test-tube. The test-tube was stoppered and shaken vigorously for about 5 min, it was allowed to stand for 30 min and observed for honeycomb froth, which was indicative of the presence of saponins.

Test for alkaloids. One gram (1 g) of the extract was dissolved in 5 mL of 10% ammonia solution and extracted with 15 mL of chloroform. The chloroform portion was evaporated to dryness and the resultant residue dissolved in 15 mL of dilute sulphuric acid. One quarter of the solution was used for the general alkaloid test while the remaining solution was used for specific tests.

Mayer's reagent (Bertrand's reagent). Drops of Mayer's reagent was added to a portion of the acidic solution in a test tube and observed for an opalescence or yellowish precipitate indicative of the presence of alkaloids.

Dragendorff's reagent. Two millilitres (2 mL) of acidic solution in the second test- tube were neutralized with 10% ammonia solution. Dragendorff's reagent was added and turbidity or precipitate was observed as indicative of presence of alkaloids.

Tests for carbohydrate (Molisch's test). A few drops of Molischs solution was added to 2 mL of aqueous solution of the extract, thereafter a small volume of concentrated sulphuric acid was allowed to run down the side of the test tube to form a layer without shaking. The interface was observed for a purple colour as indicative of positive for carbohydrates.

Tests for carbohydrate (Barfoed's test). One milliliter (1 mL) of aqueous solution of the extract and 1ml of Barfoed's reagent were added into a test-tube, heated in a water bath for about 2 min. Red precipitate showed the presence of monosaccharaides.

Standard test for free reducing Sugar (Fehling's test). Two milliliters (2 mL) of the aqueous solution of the extract in a test tube was added into 5 mL mixture of equal volumes of Fehling's solutions I and II and boiled in a water bath for about 2 min. The brick-red precipitate was indicative of the presence of reducing sugars.

Test for cardiac glycosides. Two millilitres (2 mL) of the aqueous solution of the extract was added into 3 drops of strong solution of lead acetate. This was mixed thoroughly and filtered. The filtrate was shaken with 5 mL of chloroform in a separating funnel. The chloroform layer was evaporated to dryness in a small evaporating dish. The residue was dissolved in a glacial acetic acid containing a trace of ferric chloride; this was transferred to the surface of 2 mL concentrated sulphuric acid in a test tube. The upper layer and interface of the two layers were observed for bluish-green and reddish-brown colouration respectively as indicative of the presence of cardiac glycosides.

Test for steroids (Liebermann-Burchard's test). The amount of 0.5 g of the extract was dissolved in 10 mL anhydrous chloroform and filtered. The solution was divided into two equal portions for the following tests. The first portion of the solution above was mixed with one ml of acetic anhydride followed by the addition of 1 mL of concentrated sulphuric acid down the side of the test tube to form a layer underneath. The test tube was observed for green colouration as indicative of steroids.

Test for steroids (Salkowski's test). The second portion of solution above was mixed with concentrated sulphuric acid carefully so that the acid formed a lower layer and the interface was observed for a reddish-brown colour indicative of steroid ring.

Test for flavonoids (Shibita's reaction test). One gram (1 g) of the water extract was dissolved in methanol (50%, 1-2 mL) by heating, then metal magnesium and 5 - 6 drops of concentrated HCl were added. The solution when red was indicative of flavonols and orange for flavones.

Test for flavonoids (pew's test). Five millilitres (5 mL) of the aqueous solution of the water extract was mixed with 0.1 g of metallic zinc and 8ml of concentrated sulphuric acid. The mixture was observed for red colour as indicative of flavonols.

Test for anthraquinones (Borntrager's reaction for free anthraquinones). One gram (1 g) of the powdered seed was placed in a dry test tube and 20 mL of chloroform was added. This was heated in steam bath for 5 min. The extract was filtered while hot and allowed to cool. To the filtrate was added with an equal volume of 10% ammonia solution. This was shaken and the upper aqueous layer was observed for bright pink colouration as indicative of the presence of Anthraquinones. Control test were done by adding 10 mL of 10 % ammonia solution in 5ml chloroform in a test tube.

QUANTIFICATION OF PHYTOCHEMICALS

Determination of Alkaloid:

The sample was dissolved in dimethyl sulphoxide (DMSO), added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/g of extract.

Determination of Total flavonoid content:

Total flavonoid content was measured by the aluminium chloride colorimetric assay. The reaction mixture consists of 1 mg of sample and 4 ml of distilled water was taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was treated and after 5 minutes, 0.3 ml of 10 % aluminium chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was treated and diluted to 10 ml with distilled water. A set of reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract.

Determination of Saponin:

Total saponin determination was done using anis aldehyde reagent. Sample solution was prepared in water. Standard saponin solution, weighs 10 mg of diosgenin, dissolve in 16 mL of methanol, and add 4mL of distilled water. Standard solutions of diosegenin (20, 40, 60, 80 and 100 µg/ml) were prepared 80%aqueous methanol. Mix thoroughly and start pipetting immediately. For total saponins estimation 500 µg of sample, 500 µl of 0.5% anise aldehyde reagent, were mixed and kept aside for 10 min. Later, 2 ml of50% sulphuric acid reagent was added and tubes were mixed. Tubes were then kept in water bath with constant temperature of 60º. After 10 min tubes were cooled and absorbance was taken at 435 nm. Same method was followed for standard also. The amount of saponins was calculated as saponin equivalent from the calibration curve of standard.

Determination of Terpenoid:

Preparation of the reference solution: Linalool reference substance (10mg) was accurately weight, added in a 10ml volumetric flask, diluted with ethyl acetate to the marked line to afford a concentration of1.0mg/ml standard solution. Preparation of the test solution: The sample was precisely measured and placed in a 10ml volumetric flask, diluted with ethyl acetate to the marked line. Chromogenic method: The color developing agent applied on this experiment was prepared by the procedure as follows, 5% vanillin-acetic acid solution plus 2mL of perchloric acid were heated at 65℃ for20min, then cooled in ice water and warmed up to room temperature after being shaken. Vanillin (500mg) was dissolved in acetic acid (10ml) to prepare the vanillin solution. The standard curve 0.0,0.2,0.4,0.8,1.2,1.6,2.0 ml Linalool standard solution was precisely measured, placed in a 10 ml flask with ethyl acetate to volume marked line, The sample solution and standard mixture was then shaken, colored according to the chromogenic method. The absorbance (A) of each solution was measured at 210nm wavelength, a blank solution as the control reference.

Determination of Glycosides:

10% extract was mixed with 10mL freshly prepared Baljet's reagent (95 mL of 1% picric acid + 5 mL of 10% NaOH). After an hour, the mixture was diluted with 20 mL distilled water and the absorbance was measured at 495 nm by UV/VIS spectrophotometer. For preparation of the standard curve, 10mL of different concentrations (12.5-100 mg/L) of securidaside were prepared. Total glycosides from were expressed as mg of securidaside per gm.

Determination of total phenolic content:

The concentration of phenolics in plant extracts was determined using spectrophotometric method. Folin-Ciocalteu assay method was used for the determination of the total phenol content. The reaction mixture consists of 1 ml of extract and 9 ml of distilled water was taken in a volumetric flask (25 ml). One milliliter of Folin-Ciocalteu phenol reagent was treated to the mixture and shaken well. After 5 minutes, 10 ml of 7% Sodium carbonate (Na_2CO_3) solution was treated to the mixture. The volume was made up to 25 ml. A set of standard solutions of gallic acid (20, 40, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Incubated for 90 min at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an Ultraviolet (UV) /Visible spectrophotometer. Total phenol content was expressed as mg of GAE/g.

Determination of tannin Content:

The tannins were determined by Folin - Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35 % Na_2CO_3 solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of Tannic acid (20,40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of Tannic acid /g.

Estimation of Steroids:

1ml of sample extract and different concentration of Prednisone standard steroid solution was transferred into 10 ml volumetric flasks.2ml of 4N Sulphuric acid was added and 2ml of 0.5% of iron (III) chloride was added followed by 0.5mlof 0.5% potassium hexa cyanoferrate (III) solution. The mixture was heated in a water-bath maintained at 70 ± 20^0 C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank. Total Steroid content was expressed as mg of Prednisone/gm.

LC MS analysis of the leaf extract:
Version : 6200 series TOF/6500 series, Q-TOF B.05.01 (B5125.3)
Acq method: Metabolite ESI +ve MSMS and Metabolite ESI -ve MSMS
Instrumental parameters

Component Name Ion Source Can wait for temp.	MS Q-TOF Dual AJS ESIEnable	Component Model Stop Time (min)Fast Polarity	G6550A 30.00 N/A
MS Abs. threshold	200	MS Rel. threshold(%)	0.010
MS/MS Abs. threshold	5	MS/MS Rel. threshold(%)	0.010
Tune File	AutoTune (3).tun		

Flow	0.300 mL/min
Use Solvent Types	Yes
Stroke Mode	Synchronized
Low Pressure Limit	0.00 bar
High Pressure Limit	1200.00 bar
Max. Flow Ramp Up	100.000 mL/min²
Max. Flow Ramp Down	100.000 mL/min²
Expected Mixer	No check

Solvent Composition

	Channel	Ch. 1 Solv.	Name 1	Ch2 Solv.	Name 2	Selected	Used	Percent
1	A	100.0 % WaterV.02	0.1% FA in water	100.0 % WaterV.02	0.1% FA in water	Ch. 2	Yes	95.00 %
2	B	100.0 % Methanol V.03		100.0 % Acetonitrile V.02		Ch. 2	Yes	5.00 %

RESULTS AND DISCUSSIONS

Table 1: Qualitative determination of phytochemicals in hydro-alcoholic extract of *Passiflora quadrangularis* leaves

SL NO	CHEMICAL CONSTITUENTS	HYDRO-ALCOHOLIC EXTRACT OF LEAF
1	Carbohydrates	+
2	Steroids	+
3	Glycosides	+
4	Flavanoids	+
5	Tannins	+
6	Phenols	+
7	Proteins	+
8	Alkaloids	+

Table 2: Phytochemical quantification of hydro-alcoholic extract of leaves of *Passiflora quadrangularis*

SL NO	NAME OF THE TEST	RESULTS	TEST METHOD
1	Tannin	28.5 mg/g	By UV-Visible spectrophotometric method
2	Alkaloids	140 mg/g	
3	Phenolic compounds	305 mg/g	
4	Steroids	1.7 mg/g	
5	Flavonoids	296 mg/g	
6	Glycoside	40 mg/g	
7	Terpenoids	9.3 mg/g	
8	Saponins	107 mg/g	

Figure 1: Phytochemical Quantification of phytochemicals in hydroalcoholic extract of leaf of *Passiflora quadrangularis*

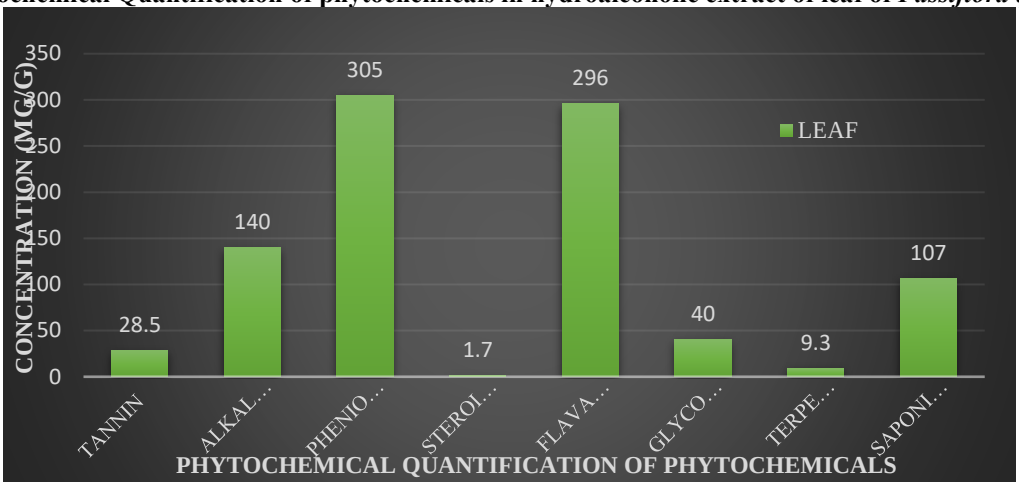

Table no 3: Compound table of (M+H) ionization method

Compound Label	RT	Mass	Abund	MFG Formula	DB Formula	DB Diff (ppm)	Hits (DB)
Compound 1	0.953						
Cpd 2: D-1-Aminopropan-2-ol O-phosphate; C3 H10 N O4 P	1.175	155.0325	33427	C3 H10 N O4 P	C3 H10 N O4 P	14.16	8
Cpd 3: Retronecine; C8 H13 N O2	1.246	155.0933		C8 H13 N O2	C8 H13 N O2	8.66	3
Cpd 4: Fenazaquin; C20 H22 N2 O	1.533	306.1769		C20 H22 N2 O	C20 H22 N2 O	-12.09	1
Cpd 5: Retronecine; C8 H13 N O2	1.596	155.0935		C8 H13 N O2	C8 H13 N O2	7.59	3
Cpd 6: Neuraminic acid; C9 H17 N O8	1.604	267.0939		C9 H17 N O8	C9 H17 N O8	5.53	7
Compound 7	1.605						
Cpd 8: Pirbuterol; C12 H20 N2 O3	1.788	240.1449		C12 H20 N2 O3	C12 H20 N2 O3	10.42	1
Compound 9	1.881						
Cpd 10: Fenazaquin; C20 H22 N2 O	1.908	306.1753	22939	C20 H22 N2 O	C20 H22 N2 O	-6.89	1
Cpd 11: Retronecine; C8 H13 N O2	1.972	155.0933		C8 H13 N O2	C8 H13 N O2	8.79	3
Compound 12	2.032						
Cpd 13: Eupacunolin; C22 H28 O8	2.088	420.1753	143872	C22 H28 O8	C22 H28 O8	7.41	8
Cpd 14: Dihydromethylsterigmatocysti	2.209	342.0784		C18 H14 O7	C18 H14 O7	-13.13	1
Compound 15	2.391						
Cpd 16: Poly-g-D-glutamate; C20 H30 N4 O12	3.5	518.1874		C20 H30 N4 O12	C20 H30 N4 O12	-2.72	4

Cpd 17: D-Tryptophan; C11 H12 N2 O2	3.611	204.0881		C11 H12 N2 O2	C11 H12 N2 O2	8.69	8
18: Hydroxypropyl methyl cellulose; C25 H44 O21	3.613	680.2398		C25 H44 O21	C25 H44 O21	-3.4	3
pd 19: 5alpha-Ethoxy-6beta- hydroxy-5,6-dihydrophysalin B; C30 H36 O11	3.886	572.2182		C30 H36 O11	C30 H36 O11	13.25	1
Cpd 20: Pyranomammea B; C22 H28 O6	3.959	388.1846		C22 H28 O6	C22 H28 O6	10.4	10
Cpd 21: 4'-tert-Butyl-2',6'-dimethyl-3',5'-dinitroacetophenone; C14 H18 N2 O5	4.345	294.1195	399577	C14 H18 N2 O5	C14 H18 N2 O5	6.94	10
Cpd 22: Pentobarbital; C11 H18 N2 O3	4.404	226.1324	490054	C11 H18 N2 O3	C11 H18 N2 O3	-3.04	3
Cpd 23: Fluocinolone acetonide; C24 H30 F2 O6	4.706	452.1999		C24 H30 F2 O6	C24 H30 F2 O6	2.44	4
Cpd 24: Pilocarpine; C11 H16 N2 O2	4.824	208.1216		C11 H16 N2 O2	C11 H16 N2 O2	-2.19	4
Cpd 25: Fluocinolone acetonide; C24 H30 F2 O6	5.066	452.1997		C24 H30 F2 O6	C24 H30 F2 O6	3.02	3
Cpd 26: Rutin; C27 H30 O16	5.13	610.1501		C27 H30 O16	C27 H30 O16	5.44	10
Cpd 27: Pilocarpine; C11 H16 N2 O2	5.193	208.1216		C11 H16 N2 O2	C11 H16 N2 O2	-1.95	4
Cpd 28: Kuwanon Z; C34 H26 O10	5.488	594.1547		C34 H26 O10	C34 H26 O10	-3.57	10
Cpd 29: N-Carboxyacetyl-D-phenylalanine; C12 H13 N O5	5.861	251.0769	129805	C12 H13 N O5	C12 H13 N O5	9.83	3
Cpd 30: Kaempferol 3-rhamnoside 7-xyloside; C26 H28 O14	5.961	564.1444		C26 H28 O14	C26 H28 O14	6.3	10
Cpd 31: 6-C-Galactosylluteolin; C21 H20	6.096	448.0974	235236	C21 H20 O11	C21 H20 O11	6.96	10
Cpd 32: 7-Dehydrologanin tetraacetate; C25 H32 O14	6.263	556.1779		C25 H32 O14	C25 H32 O14	2.3	10
Cpd 33: Isovitexin 2"-O- arabinoside; C26 H28 O14	6.266	564.1437		C26 H28 O14	C26 H28 O14	7.45	10
Cpd 34: Kanzonol G; C26 H30 O6	6.326	438.1992		C26 H30 O6	C26 H30 O6	11.39	3

Cpd 35: Demethyloleuropein; C24 H30 O13	6.567	526.1668	135124	C24 H30 O13	C24 H30 O13	3.55	3
Cpd 36: Genistin; C21 H20 O10	6.575	432.1025		C21 H20 O10	C21 H20 O10	7.22	10
Cpd 37: 7-Dehydrologanin tetraacetate; C25 H32 O14	6.702	556.1778		C25 H32 O14	C25 H32 O14	2.53	10
Cpd 38: Talampanel; C19 H19 N3 O3	6.849	337.1399	89968	C19 H19 N3 O3	C19 H19 N3 O3	8.15	4
Compound 39	7.15						
Cpd 40: Afzelechin; C15 H14 O5	7.155	274.0835		C15 H14 O5	C15 H14 O5	2.18	10

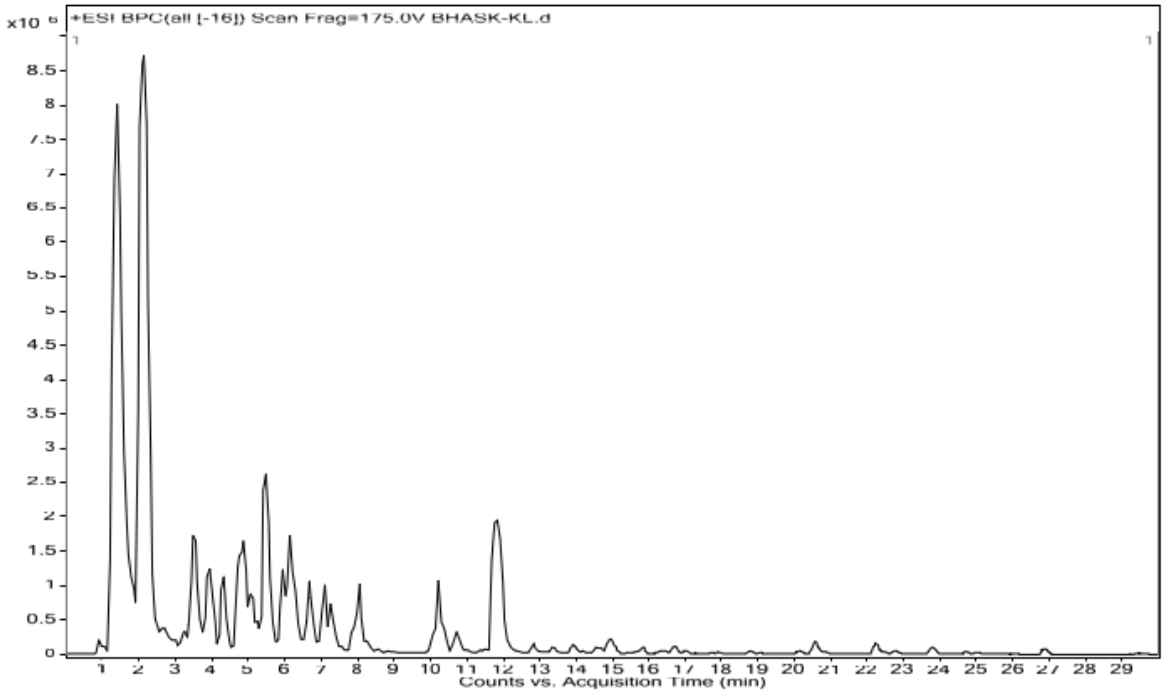

Figure 2: Chromatogram of of (M+H) ionization method

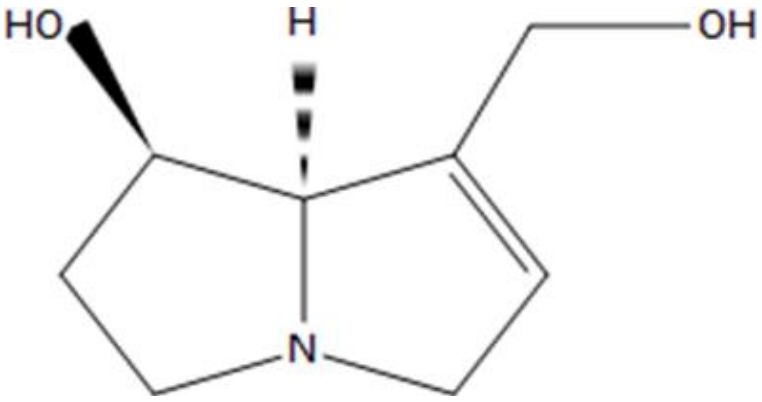

Figure 3: Molecular structure of Retronecine

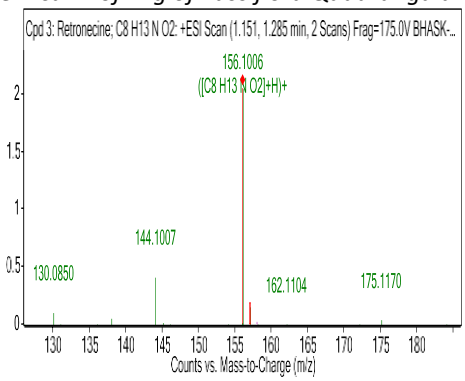

Figure 4: Mass spectrum of Retronecine

Table no 4 : Compound table of (M+H) ionization method

Compound Label	RT	Mass	Abund	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula	DB Diff (ppm)	Hits(DB)
Cpd 6: Gallic acid; C7 H6 O5	1.975	170.0221	1168			C7 H6 O5	C7 H6 O5	-3.18	
Cpd 1: Quinic acid; C7 H12 O6	2.164	192.0637	871			C7 H12 O6	C7 H12 O6	-1.51	
Cpd 10: Resorcinol; C6 H6 O2	3.084	110.0367	5217			C6 H6 O2	C6 H6 O2	0.51	
Cpd 13: Resorcinol; C6 H6 O2	3.084	110.0367	5217			C6 H6 O2	C6 H6 O2	0.51	
Cpd 16: N1-(5-Phospho-a-D-ribosyl)-5,6-dimethylbenzimidazole; C14 H19 N2 O7 P	4.54	358.0931	292749			C14 H19 N2 O7 P	C14 H19 N2 O7 P	-0.36	5
Cpd 15: C10 H12 O5	5.099	212.0686	389	212.0685	0.69	C10 H12 O5	C10 H12 O5		
Cpd 17: Quercetin 3-rhamnoside-7-glucoside; C27 H30 O16	5.114	610.1571				C27 H30 O16	C27 H30 O16	-6.17	10
Cpd 12: 1,3,6,8-Naphthalenetetrol; C10 H8	5.187	192.0421	14768			C10 H8 O4	C10 H8 O4	0.65	
Cpd 8: Chlorogenic acid; C16 H18 O9	5.377	354.0961	4077			C16 H18 O9	C16 H18 O9	-2.88	
Cpd 18: Quercetin 3,7-dirhamnoside; C27 H30 O15	5.471	594.1618				C27 H30 O15	C27 H30 O15	-5.64	10
Compound 19	5.555								
Cpd 20: Quercetin 3,7-dirhamnoside; C27 H30 O15	5.837	594.162				C27 H30 O15	C27 H30 O15	-5.98	10
Cpd 2: C8 H15 N O	5.863	141.1151	834	141.1154	-2.06	C8 H15 N O	C8 H15 N O		
Cpd 7: Vanillic acid; C8 H8 O4	5.863	168.0421	1825			C8 H8 O4	C8 H8 O4	1.15	
Cpd 14: 2,6-dihydroxybenzoic acid; C7 H6 O4	5.929	154.0269	3600			C7 H6 O4	C7 H6 O4	-2.07	
Cpd 21: Kaempferol 3-rhamnoside 7-xyloside; C26 H28 O14	6.019	564.1511				C26 H28 O14	C26 H28 O14	-5.62	10
Cpd 22: Luteolin 4'-O-glucoside; C21 H20 O11	6.085	448.1036				C21 H20 O11	C21 H20 O11	-6.78	10
Cpd 23: Daidzein 4',7-diglucoside; C27 H30 O14	6.162	578.167				C27 H30 O14	C27 H30 O14	-5.88	10

Cpd 24: Kaempferol 3-O-sophorotrioside 7-O-sophoroside; C45 H60 O31	6.253	1096.3097				C45 H60 O31	C45 H60 O31	1.98	1
Cpd 25: Daidzein 4',7-diglucoside; C27 H30 O14	6.458	578.167				C27 H30 O14	C27 H30 O14	-6.04	10
Cpd 11: C10 H10 O4	6.517	194.0579	2219	194.0579	0.05	C10 H10 O4	C10 H10 O4		
Cpd 9: C10 H10 O4	6.517	194.0579	2219	194.0579	0.05	C10 H10 O4	C10 H10 O4		
Cpd 26: Genistein 8-C-glucoside; C21 H20 O10	6.527	432.1082				C21 H20 O10	C21 H20 O10	-5.93	10
Cpd 27: Quercetin 3,7-dirhamnoside; C27 H30 O15	6.744	594.1615				C27 H30 O15	C27 H30 O15	-5.02	10
Cpd 28: Daidzein 4',7-diglucoside; C27 H30 O14	6.829	578.1664				C27 H30 O14	C27 H30 O14	-4.98	10
Cpd 29: Genistein 8-C-glucoside; C21 H20 O10	6.896	432.1079	205221			C21 H20 O10	C21 H20 O10	-5.12	10
Cpd 30: Puerarin xyloside; C26 H28 O13	7.079	548.1555				C26 H28 O13	C26 H28 O13	-4.65	4
Cpd 31: Genistein 8-C-glucoside; C21 H20 O10	7.26	432.1083				C21 H20 O10	C21 H20 O10	-6.17	10
Cpd 3: Genistein; C15 H10 O5	7.332	270.0532	734			C15 H10 O5	C15 H10 O5	-1.47	
32: Flavonol 3-O-beta-D-glucosyl-(1->2)-beta-D-glucoside; C27 H30 O13	7.876	562.1666	12799			C27 H30 O13	C27 H30 O13	3.58	7
Cpd 33: Genistein 8-C-glucoside; C21 H20 O10	7.991	432.1079				C21 H20 O10	C21 H20 O10	-5.21	10
34: Flavonol 7-O-beta-D-glucoside; C21 H20 O9	7.993	416.1128				C21 H20 O9	C21 H20 O9	-4.98	10
35: Flavonol 3-O-beta-D-glucosyl-(1->2)-beta-D-glucoside; C27 H30 O13	8.174	562.1714				C27 H30 O13	C27 H30 O13	-4.82	6
Cpd 4: alpha-licanic acid; C18 H28 O3	15.663	292.2044	1074			C18 H28 O3	C18 H28 O3	-1.79	
Cpd 5: 9-HOTE; C18 H30 O3	17.034	294.2196	6836			C18 H30 O3	C18 H30 O3	-0.52	

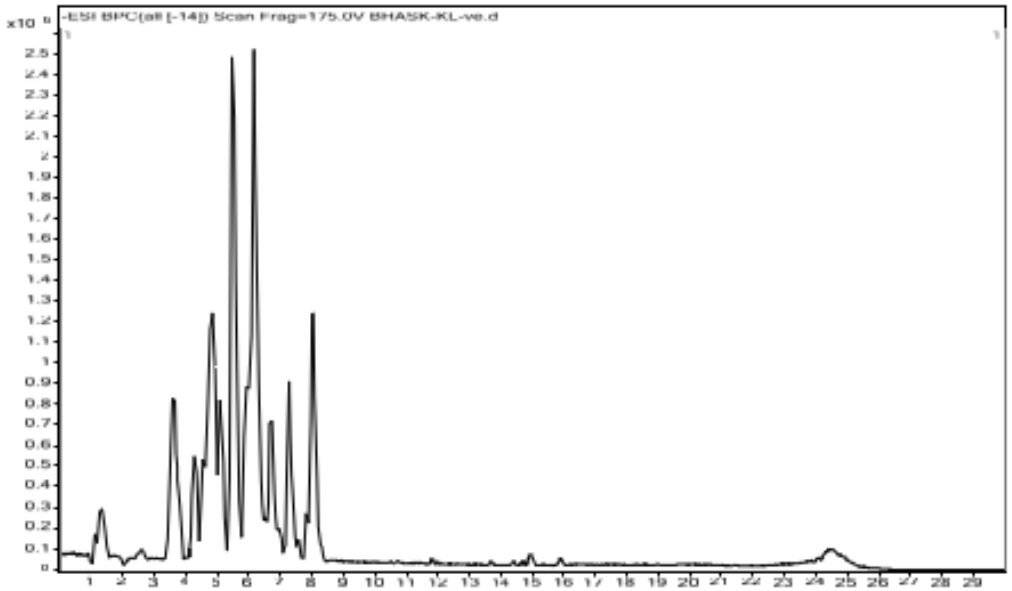

Figure 5: Chromatogram of of (M-H) ionization method

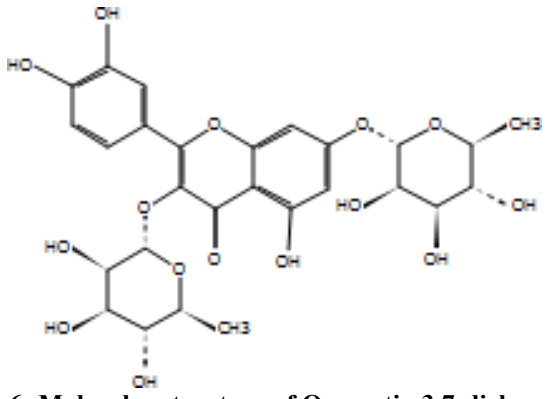

Figure 6: Molecular structure of Quercetin 3,7-dirhamnoside

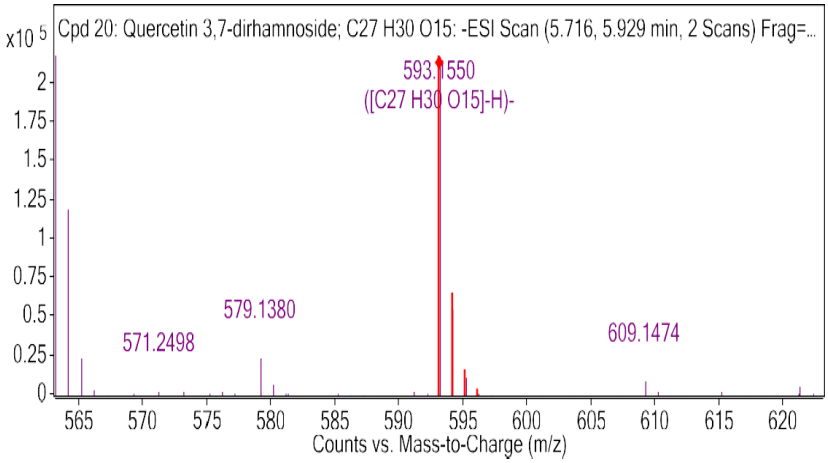

Figure 7: Mass spectrum of Quercetin 3,7-dirhamnoside

CONCLUSION

The analysis revealed the presence of alkaloids, phenolic compounds, amino acids, dicarboxylic acids, flavonoids, and glycosides. Among them, Retronecine was found to be the most prevalent. The negative mode largely accentuated the presence of flavonoid and isoflavonoid glycosides, polyphenols, fatty acid derivatives, and phenolic compounds. Among them, Quercetin 3,7-dirhamnoside was found to be the most abundant. The presence of Kaempferol 3-rhamnoside 7-xyloside was identified in both modalities. The wide range of discovered chemicals highlights the considerable pharmacological potential of Passiflora quadrangularis extracts for therapeutic uses

REFERENCES

Abubakar A. R, Haque M, (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *J. Pharm. Bioallied Sci.* 12 (1), 1–10.

Barbosa Santos T, De Araujo F, Neto A, De Freitas, (2021): Phytochemical compounds and antioxidant activity of the pulp of two brazilian passion fruit species: *Passiflora* cincinnata mast. And *Passiflora edulis* Sims. *International Journal of Fruit Science*. 21(1), 255–269.

Da Silva D, Peixoto J, Souza M, Gelape F, Silva K, de Deus R, (2017). Agronomic descriptors and ornamental potential of passion fruit species. *Ornam. Hort*. 23, 357–362.

Johnson M, Maridass M, Irudayaraj V, (2014): Preliminary phytochemical and anti-bacterial studies on Passiflora edulis. *Ethnobotanical Leaflets*. 12, 425-432.

Khandewal K R, (2005): Practical Pharmacognosy Techniques and experiments. Nirali Prakashan, Pune.

Kokate C. K, (2002); Practical Pharmacognosy. Vallabh Prakashan, Pune.

Kokate C. K, Purohit P. S, Gokhale B; (1995); Methods of crude drug evaluation pharmacognosy. Nirali Prakashan, Pune.

Leite I. B, MagalhãesC. D, Monteiro M, Fialho E, (2021): Addition of honey to an apple and passion fruit mixed beverage improves its phenolic compound profile. *Food*. 10(7), 1525.

Madhavan Azhagu S, Sa V, Ra S, Sb R, (2021). Phytochemical Screening and GC–MS Analysis of Bioactive Compounds Present In Ethanolic Leaf Extract Murraya koenigii. *Bull. Env. Pharmacol. Life Sci*. 10, 158–164.

Ramaiya S. D, Japar S. B, Muta Z. H, (2018): Nutritive Values of Passion Fruit (Passiflora species) Seeds and Its Role in Human Health. *Journal of Agriculture Food and Development.* 4, 23-30.

Raman N, (2006); Phytochemical techniques. Publishing agency New India publications; 1; New Delhi.

Suleria H. A, Barrow C. J, Dunshea F. R, (2020): Screening and characterization of phenolic compounds and their antioxidant capacity in different fruit peels. *Food*. 9(9), 1206.

Tavassoli S, Djomeh Z E, (2011): Total phenols, antioxidant potential and antimicrobial activity of methanol extract of

rosemary (Rosmarinus officinalis L.). *Global Veterinaria*. 7(4), 337–341.
Trease G. E, Evans W. C, Pharmacognosy; (1985); 12; English Language Book Society, Bailliere Tindall, London.
Zhong B, Robinson N. A, Warner R. D, Barrow C. J, Dunshea F. R, Suleria H. (2020): LC-ESI-QTOF-MS/MS characterization of seaweed phenolics and their antioxidant potential. *Marine Drugs*. 18(6), 331.