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Analysis of bioactive compounds in *Physalis minima* leaves using GC MS, HPLC, UV-VIS and FTIR techniques

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Abstract
The bioactive components of *Physalis minima* leaves have been evaluated using GCMS, HPLC, UV VIS and FTIR. The chemical compositions of the extract of *Physalis minima* leaves were investigated using Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched by the National Institute of Standards and Technology (NIST) library. GC/MS analysis of extract of *Physalis minima* leaves revealed the existence of Heneicosanoic acid (25.22), Bicyclo [4.1.0] Hepta-2, 4-dien (27.41) Octadecanoic acid (CAS), Stearic acid (31.19) and Octadeca-9, 12-dienoic acid (32.02). HPLC profiles of *Physalis minima* reported to contain four phenolic compounds, namely Ellagic acid (4.13 min), Catechol (3.59 min), Gallic acid (4.12 min) and Catechin (7.41 min). The UV- VIS profile showed the peaks at 315.09 nm, 408.09 and 676.50 nm with the absorption 0.247, 0.106 and 0.003 respectively. The results of FTIR analysis confirmed the presence of phenol, alkanes, aldehyde, secondary alcohol, amino acid, aromatic amines and halogen compound. The results of this study offer a platform of using *Physalis minima* leaves as herbal alternative for various diseases.

Keywords: GC MS, HPLC, UV-VIS, FTIR, *Physalis minima.*

1. Introduction
Medicinal plants are assuming greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase in demand in international trade because of very effective, cheaply available, supposedly have no side effects and used as an alternative to allopathic medicines. Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments [1]. Phytochemicals simply means plant chemicals. “Phyto” is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. In addition, these compounds may be responsible for the beneficial effects of fruits and vegetables on an array of health related measures [2]. Chemical principles from natural sources have become much simpler and have contributed significantly to the development of new drugs from medicinal plants [3]. The valuable medicinal properties of different plants are due to presence of several constituents i.e. saponins, tannins, alkaloids, alkenyl phenols, glycol-alkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters [4]. Among them some are act as synergistic and enhance the bioactivity of other compounds.
Within a decade, there were a number of dramatic advances in analytical techniques including HPLC, UV, FTIR, NMR and GC-MS that were powerful tools for separation, identification and structure determination of phytochemicals (Roberts and Xia, 1995) [5]. The aim of this study is to determine the bioactive compounds present in the *Physalis minima* leaves extract with the aid of GC MS, HPLC, UV-VIS and FTIR Techniques, which may provide an insight in its use of tradition medicine.

2. Materials and Methods
2.1 Plant materials
The mature *Physalis minima* leaf was collected in May 2014 from Vaduvur, Thiruvarur District, Tamil Nadu, India. The leaves were identified and authenticated by Botanist, Prof. Dr. Kulothugan, Department of Botany and Microbiology, A.V.V.M. Sri pushpam College (Autonomous), Poondi, Thanjavur District, Tamil Nadu. India. A Voucher specimen (No.

SPCH126) has been deposited in the department.

2.2 GC MS Analysis

GC MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0..32mm, column length is 30 m, column thickness 0.50 µm), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 270 ºC; ion-source temperature 200 ºC. The oven temperature was programmed from 40 ºC (isothermal for 2 min), with an increase of 8 ºC/min, to 150 ºC, then 8 ºC/min to 250 ºC, ending with a 20 min isothermal at 280 ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 51.25 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMass Ver 5.2.0 [6].

2.3 Identification of components

Interpretation on GCMS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained [7].

2.4 UV and FTIR Spectroscopic analysis

The extracts were examined under visible and UV light for proximate analysis. For UV and FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatman No. 1 filter paper by using a high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 260-900 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 400-4000 cm^{-1} and their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

2.5 HPLC Analysis

Sample preparation: The sample was prepared according to the procedure. The extraction was carried out using 2 ml of fermented broth with 50 mL of 95% ethanol under 80 KHz, 45 °C in ultrasonic extraction device for 30 min, repeated twice. The extract was collected and filtered; the filtrate was dried at 50 °C under reduced pressure in a rotary evaporator. The dried crude extract was dissolved in the 100 ml mobile phases. After filtering through a filter paper and a 0.45 mm membrane filter (Millipore), the extract was injected into HPLC.

HPLC conditions: Flavonoids were analysed using an RP-HPLC method [8], Shimadzu Corp., Kyoto, consisting of a LC-10ATVp pump, SCL 10A system controller and a variable Shimadzu SPD- 10ATVp UV VIS detector and a loop injector with a loop size of 20 µl. The peak area was calculated by a CLASSVP software. Reverse-phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250×4.6 mm i.d., particle size 5 µm, Luna 5 µ C-18; phenomenex, Torrance, CA, USA) at 25°C. The gradient elution of solvent A [water-acetic acid (25:1 v/v)] and solvent B (methanol) had a significant effect on the resolution of compounds. As a result, solvent gradients were formed, using dual pumping system, by varying the proportion of solvent A [water-acetic acid (25:1, v/v)] to solvent B (methanol). Solvent B was increased to 50% in 4 min and subsequently increased to 80% in 10 min at a flow rate of 1.0 mL/min. Detection wavelength was 280 nm.

3. Results and Discussion

The pharmacological activities of any plant sample are due to the presence of metabolites, secondary metabolites and secretory products in it. These usually consist of the phenolic compounds, alkaloids, tannins, saponins, carbohydrates, glycosides, flavonoids, steroids, etc. Most phenolic compounds such as flavonoids, glycosides, triperinoids, flavonons, carbohydrates and anthraquinones are found distributed throughout the plant kingdom [9]. Similarly, the polyphenolic compounds most commonly found in plant extracts are the phenolic acids, flavonoids and tannins [10]. These compounds together with other phenolic structures of plant origin have been reported as scavengers of Reactive Oxygen Species (ROS) and are seen as promising therapeutic drugs for free radical mediated pathologies including diabetic, cardiovascular diseases [11]. Most flavonoidic compounds exhibit antipyretic, analgesic, anti-inflammatory, anti-arthritic, antioxidant and immuno-modulatory properties [12, 13]. These activities of flavonoidic compounds may be due to the presence of gallic acid, ellagic acid, quercitin, tannin acid, vanillin, resorcinol, catechin, etc.

3.1 GC-MS Analysis

Thirty one compounds was identified in *Physalis minima* leaves by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 1 and Fig 1). The prevailing compounds where Heneicosanoic acid (25.22), Bicyclo [4.1.0] Hepta-2, 4-dien (27.41) Octadecanoic acid (CAS), Stearic acid (31.19) and Octadeca-9, 12-dienoic acid (32.02). In the present study thirty one chemical constituents have been identified from extract of the plant of *Physalis minima* by Gas Chromatogram- Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results.

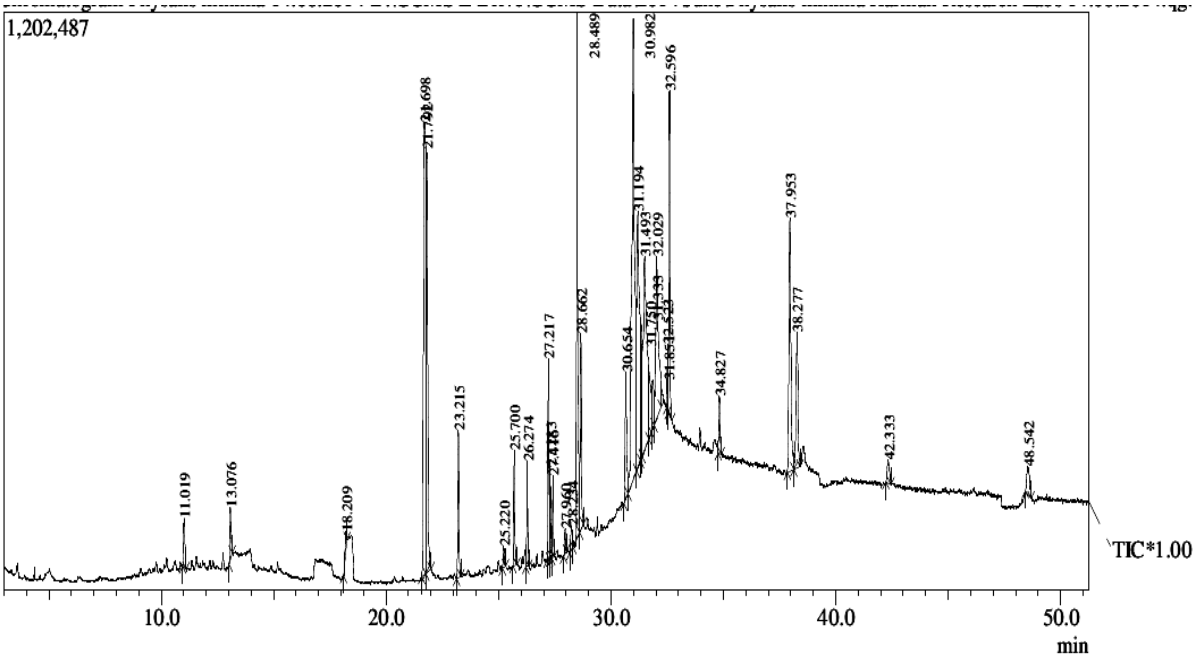

Fig 1: GC MS analysis of *Physalis minima* leaf extract

Table 1: GC MS analysis of *Physalis minima* leaf extract

Peak	R .Time	Area%	Molecular Formula	Name
1	11.019	0.64	C_{14} H_{30}	Tetradecane (CAS) n-Tetradecane $$
2	13.076	0.62	C_{12} H_{36} O_6 Si_6	Cyclohexasiloxane, dodecamethyl-
3	18.209	0.28	$C_{14}H_{42}O_7Si_7$	Cycloheptasiloxane, Tetr
4	21.698	12.02	C_{12} H_{14} O_4	1,2-Benzenedicarboxylic acid,diethyl e
5	21.792	8.24	C_8 H_6 N_4 O_5	2,4-Imidazolidinedione, 1-[[(5-nitro-
6	23.215	2.01	$C_{16}H_{48}O_8Si_8$	Cyclooctasiloxane, Hexad
7	25.220	0.28	$C_{21}H_{42}O_2$	Heneicosanoic acid $$ Heni
8	25.700	1.26	$C_{16}H_{22}O_3Si_3$	1,3-Diphenyl-1,3,5,5-Tetramet
9	26.274	1.05	C_{18} H_{54} O_9 SI_9	Octadecamethylcyclonona
10	27.217	2.26	C_{18} H_{24} O_6	Phthalic acid, butyl ester, ester with b
11	27.283	1.03	C_{14} H_{14} N_2 O	2-Pyridinepropanamide, N-P
12	27.416	0.77	C_{27} H_{32}	Bicyclo[4.1.0]Hepta-2,4-dien,
13	27.960	0.32	C_{16} H_{22} O_4	1,2-Benzenedicarboxylic acid, bis(2-m
14	28.234	0.39	C_{25} H_{42} O_2	Cyclopropanebutanoic acid, 2-[[2-[[2-
15	28.489	7.30	$C_{15}H_{30}O_2$	Pentadecanoic acid $$ 14FA
16	28.662	3.02	C_{16} H_{22} O_4	1,2-Benzenedicarboxylic acid, butyl 2-
17	30.654	2.07	C_{21} H_{22} FE N_2 O_5	Iron, monocarbonyl-(1,3-butadiene-1,4
18	30.982	15.69	C_{19} H_{34} O	(Z,Z)-6,9-CIS-3,4-EPOXY-NONAD
19	31.194	7.19	C_{18} H_{36} O_2	Octadecanoic acid (CAS) Stearic acid
20	31.333	2.03	C_{18} H_{34} O_2	9-Octadecynenitrile (CAS)
21	31.493	8.10	C_{20} H_{36} O_2	Ethyl linoleate $$ Linoleic acid, E
22	31.750	1.35	C_{15} H_{15} N	1,3,4-Trimethylcarbazole $$
23	31.851	1.17	C20 H38 O2	Ethanol, 2-(9,12-octadecadienyloxy)-,
24	32.029	4.97	C18 H32 O2	Octadeca-9,12-dienoic acid
25	32.523	0.71	C18 H54 O9 SI9	Octadecamethylcyclonon
26	32.596	4.00	C12 H13 N3 O S	4-p-chorophenyl-2-dimethylamino-5-n
27	34.827	0.84	C12 H10 F N5	1H-Purin-6-amine, [(2-fluorophenyl)me
28	37.953	5.94	C24 H38 O4	1,2-Benzenedicarboxylic acid, bis(2-e
29	38.277	2.97	C24H38O4	Bis(2-ethylhexyl) phthalate
30	42.333	0.60	NIL	Silikonfett SE30 (GREVELS)
31	48.542	0.86	C30 H50 O	1,6,10,14,18,22-Tetracosahexa

3.2 Determination of HPLC retention times

HPLC profiles of *Physalis minima* were analysed and four phenolic compounds namely Ellagic acid (4.13 min), Catechol (3.59 min), Gallic acid (4.12 min) and Catechin (7.41 min), having different elution times could be obtained (Figure 2 and Table 2) when each compound was analyzed

individually using the mobile gradient phase consisting of methanol and 1% acetic acid in water during 30 minutes run time. Earlier review of [14 -17] supported the findings of these compounds.

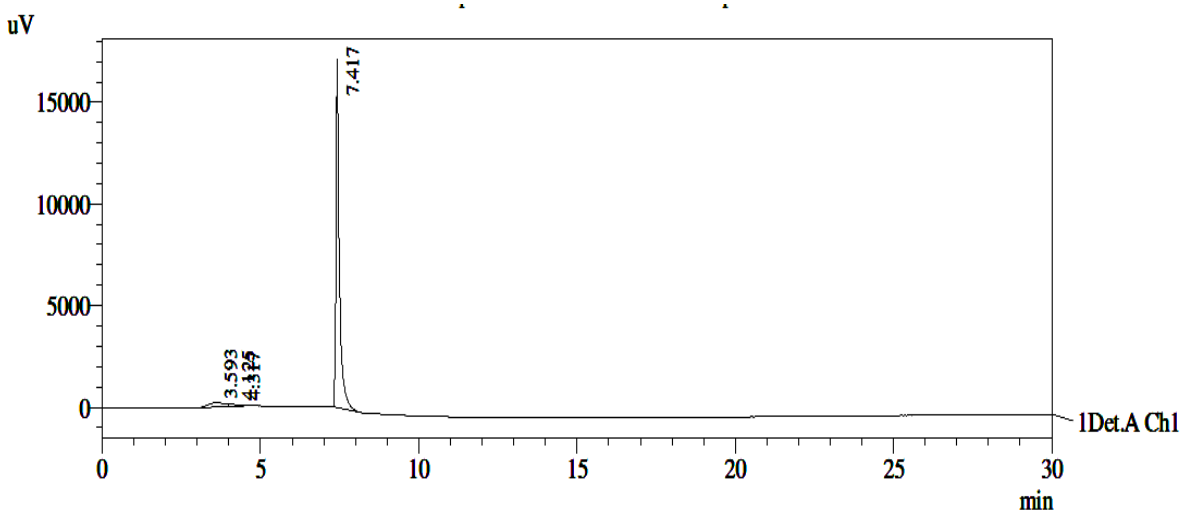

Fig 2: HPLC analysis of *Physalis minima* leaf extract

Table 2: HPLC analysis of *Physalis minima* leaf extract

Peak	Area %	Retention Time	Literature (RT)	Name of the compound
1.	5.670	3.593	3.60	Catechol
2.	0.863	4.125	4.12	Gallic acid
3.	0.830	4.312	4.43	Ellagic acid
4.	92.63	7.41	7.00	Catechin

3.3 Spectrophotometric analysis

The UV-VIS profile of plant extract was taken at the 200 to 800 nm wavelength due to the sharpness of the peaks and proper baseline. The UV-visible spectra were performed to identify the compounds containing σ- bonds, π-bonds, and lone pair of electrons, chromophores and aromatic rings. The profile showed the peaks at 315.09 nm, 408.09 and 676.50 nm with the absorption 0.247, 0.106 and 0.003 respectively (Fig-3 and Table 3). Occurrence of peaks at 234-676 nm reveals the presents of phenolic and alkaloids in the *Physalis minima.* On comparison of the spectra of seeds and flowers, shows that the extract has some similar alkaloid, flavonoids, and glycosides compounds reported [18, 19].

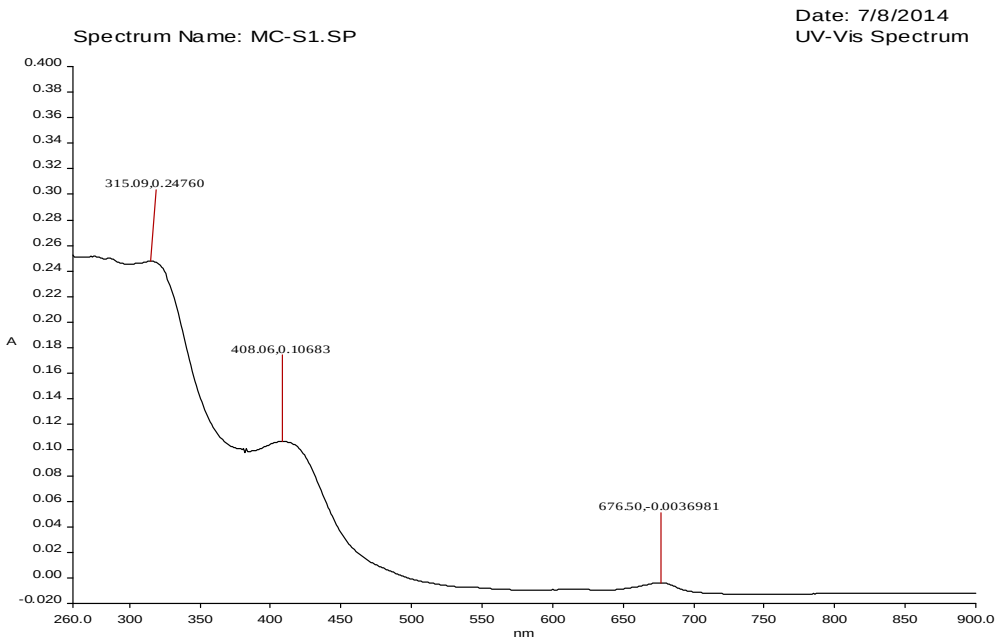

Fig 3: UV-Vis Spectral analysis of *Physalis minima* leaf extract

Table 3: UV-VIS Peak Values of Extract of *Physalis minima* leaf

S. No.	Wave length (nm)	Absorption Peak
1	315.09	0.247
2	408.06	0.106
3	676.50	0.003

3.4 Functional groups identification

The FTIR spectrum was used to identify the functional groups of the active components present in extract based on the peaks values in the region of IR radiation. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of phenol, alkanes, aldehyde, secondary alcohol, amino acid, aromatic amines and halogen compound (Fig- 4, and Table- 4).

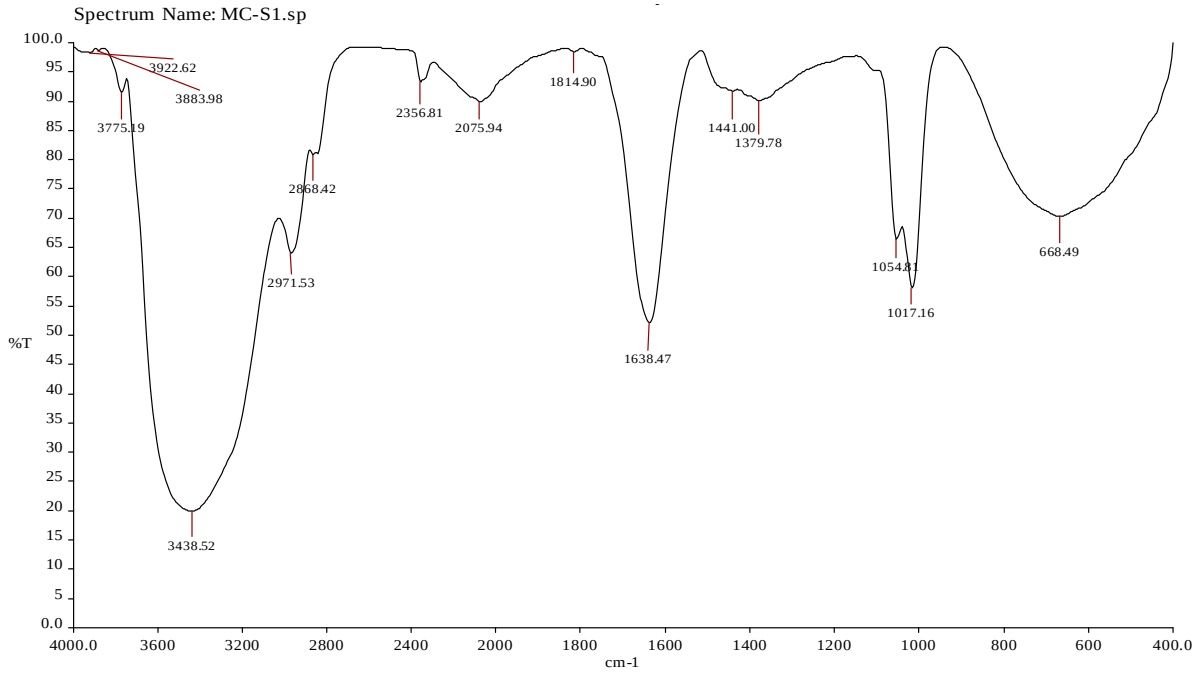

Fig 4: FTIR analysis of *Physalis minima* leaf extract

Table 4: FTIR Peak Values of Extract of *Physalis minima* leaf extract

S. No.	Peak Values	Functional groups
1	3922.62	Unknown
2	3883.98	Unknown
3	3775.19	Phenols
4	3438.52	Amides
5	2971.53	Alkanes
6	2858.42	Aldehydes
7	2356.81	Unknown
8	2075.94	Amino acids
9	1814.90	Unknown
10	1638.47	Alkenes
11	1441.00	Unknown
12	1379.78	Alkanes
13	1054.82	Carboxylic acid
14	1011.16	Alcohol
15	668.49	Halogen compound

4. Conclusion

GC/MS results signified the presence of thirty one phytochemical constituents. The prevailing compounds were Heneicosanoic acid, Bicyclo [4.1.0] Hepta-2, 4-dien, Octadecanoic acid (CAS), Stearic acid and Octadeca-9, 12-dienoic acid. HPLC analysis provided a good platform for identification and quantification of four phenolic compounds as Ellagic acid, Catechol, Gallic acid and Catechin present in *Physalis minima* leaves. The UV- VIS profile showed the peaks at 315.09 nm, 408.09 and 676.50 nm respectively. The results of FTIR analysis confirmed the presence of phenol, alkanes, aldehyde, secondary alcohol, amino acid, aromatic amines and halogen compound. The results of this study offer a platform of using *Physalis minima* leaves as herbal alternative for various diseases including diabetic, cardiovascular etc.

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