



Comparative Analysis of Bioactive Compounds and Anticancerous Activities in Leaf and Stem Extract of *Physalis minima*

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Abstract

Physalis minima, commonly known as native gooseberry is a perennial herb which grows mostly in the tropical regions and is used in the traditional medicines as a curative for gastric ulcers and also for respiratory tract related ailments. The leaf and stem crude extract was separated by Soxhlet using chloroform as solvent followed by the phytochemical screening to identify the presence of secondary metabolites. The Fourier-transform infrared spectroscopy (FTIR) showed the presence of various functional group such as phenol, alkane, aldehyde, alkyls, amino acid, aromatic amino acids and secondary alcohol. The Gas chromatography - mass spectrometry (GC-MS) result showed the presence of total of 24 bioactive compounds in both extracts such as Heptacosane, 1-chloro, Heptacosane, Octadecanoic acid, N-hexadecanoic acid and Hexatriacontane. The extract was subjected to for its anti-cancerous activity using MTT assay which had a good result against HL60 cell line and antibacterial activities.

Keywords: HL60, MTT Assay, *Physalis minima*, Phytochemical Screening, Soxhlet Extraction

1. Introduction

Physalis minima is a wild gooseberry and a pantropical annual herb. It is a perennial herb belonging to Solanaceae family. Native gooseberry, wild Cape gooseberry and pygmy ground cherry are the other common names of this plant. The fruit is edible, yellowish and encapsulated in papery cover. The fruit is a good source of vitamin C and is considered to be a diuretic, purgative and is used to relieve pain (analgesic action) and cure spleen disorder^{1,2} *Physalis minima* grows mostly in the south Asian regions, belongs to Solanaceae family. It grows to about 20–50 cm and is shaped like the tomato, making a difference that it is encapsulated inside a protective membranous covering and so in Tamil it is called Sodakkuthakkali, the latter

part of the name refers to the native tomato. The plant has its uses in the field of old medicinal practices like *Ayurveda* where the extracts of this plant in varied concentrations were used as analgesic and also as an anti-inflammatory agent. It was also proved to have some effect on the respiratory tract disease and in the gastrointestinal tract related problems. But till date the plant has not been exploited commercially for its medicinal usage, instead it still resides as a traditional medicine.

The medicinal properties in plants are due to the hundreds of phytochemicals produced by them³ Depending on their role in plant metabolism phytochemicals are classified as primary or secondary constituents. Secondary metabolism in a plant helps the plant to survive in its environment¹. The plant was

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used in traditional medicine as a curative for almost all the disorders before the advent of synthetically derived medicines. The presence of several compounds such as saponins, tannins, alkaloids, alkenyl phenols, glycolalkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters^{4,5} result in the increased defense mechanism of the plant. It is also useful for mankind as a medicine for various ailments. This study involves the anticancerous and also the antimicrobial activities of *Physalis minima*. Analytic techniques such as FTIR was done to identify the phytochemicals present in the plant. Cell line studies in carcinoma cell was done for in-vitro studies and antimicrobial assay was also performed to determine its potential against microbial attack.

2. Methodology

2.1 Sample Collection

The plant *Physalis minima* were procured from the street grown plants in Tiruchirapalli district. The plant was collected and washed, then packed in a plastic cover and was brought to the Molecular diagnostics and bacterial pathogenomics laboratory.

2.2 Sample Processing

The leaves and stem of the sample plant were initially dried in sun for about 48 hrs and then it was kept in the hot air oven at 50°–60°C for 24 hours for complete drying. The plant sample after drying was subjected to powdering into small granules using a mixer and these were separated by sieving.

2.3 Compound Extraction

The powdered samples were subjected to extraction process for obtaining the crude extract to perform the experiment. The extraction process was used with chloroform as solvent and with the Soxhlet apparatus as the extraction equipment used, since the compounds to be taken for study (flavonoids, alkaloids, etc.) are heat stable. The temperature for the extraction was set at 60°C, since the boiling point of chloroform is around 60.2°C. The extract was obtained after seven cycles of evaporation and condensation. The extract

was concentrated to 10ml by evaporating it at 62°C in a water bath and a part of it was used for further analysis. The remaining portion was evaporated completely to obtain a sticky paste which was sent for cytotoxic studies.

2.4 Phytochemical Screening

The phytochemical screening was done by various tests for each phytochemical compound like alkaloids, phlobatannins, triterpenoids, flavonoids, lipids, steroids and terpenoids⁵.

2.5 Antibacterial Assay

The Chloroform extracts of *Physalis minima* were tested for their resistance against certain strains of bacteria. The plant extracts were subjected to antimicrobial sensitivity test by exposing the extract to specific strains of bacteria. The bacterial strain was revived from the glycerol stock. The antimicrobial assay was performed by following the agar plate Well Diffusion method. There were four wells made on the surface of each petridish, a positive control, a negative control, the extracts obtained were put into the four wells. The positive control was Ampicillin and as negative control; chloroform was used⁶. The petridishes were sealed with paraffin wax and were incubated at 37°C for 24 hours. The next day, zone of inhibition was recorded for each strains of bacteria and its reactivity to the leaf extract of *Physalis minima*. The concentration of the samples (leaf and stem chloroform extract) was optimized to 1.4 µg/µl for leaf and 1.19 µg/µl for stem extract respectively. The Ampicillin was concentrated to 0.1 g/ml.

2.6 Fourier Transforms Infrared Spectroscopy

Fourier Transforms Infrared Spectroscopy is the technique by which the specific functional group in a compound given out for analysis. FTIR is used to obtain an infrared beam absorption or emission spectrum in a solid, liquid, or gas. FTIR spectrophotometer makes use of the infrared beams that will be passed through the sample in a specified manner and collects high-spectral-resolution data over a wide spectral range.

In FTIR spectrophotometer, the beam is emitted from the source at different combination of wavelength

and is allowed to pass through the sample. In this first beam, some wavelength gets absorbed, followed by other wavelengths of different frequency combinations and again the same process is repeated. At last we obtain a FTIR interferogram, the raw data which is given out in the form of graphical representation.

For this study, FTIR for powdered leaf sample of *Physalis minima* was done using FTIR spectrophotometer (4000cm^{-1} – 400cm^{-1}) with KBr disc used as the binding agent. The interferogram was obtained and the results were interpreted⁷.

2.7 GC-MS

The samples were subjected to GC-MS analysis to detect the presence of bioactive components. The Clarus 680 (GC model) and Clarus 600 (EI) (MS model) with Turbo Mass ver 5.4.2 software was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, $30\text{ m} \times 0.25\text{ mm ID} \times 250\mu\text{m df}$) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The $1\mu\text{L}$ of extract sample injected into the instrument and the oven temperature was as follows: 60°C for 2 min followed by 300°C at the rate of $10^\circ\text{C min}^{-1}$ and 300°C , where it was held for 6 min. The mass detector conditions were: transfer line temperature 240°C ; ion source temperature 240°C ; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

2.8 Cytotoxicity Test

Some of the compounds present in the plants act against the growth and development of cancer cells that is they possess anticancerous activities which are exhibited by the secondary metabolites produced by the metabolic activities of the plant. According to the literature review for *Physalis minima* taken for study, some of the scientists who had performed *invitro* experiments to test for the anti-cancerous activities of this plant extract, have proven that the chloroform crude leaf extract of *Physalis minima* had been proven to kill the NCI-H23 type of cancer cell strain, which

is an adenomatous lung cancer cell line. The results have been turned up to show 90% positive results when tested with NCI-H23 cell line^{5,7}. The *invitro* studies to test for anti-cancerous activity of *Physalis minima* has been carried out using the HL-60 cell line, which causes the normal Lung cancer in humans. The experiment was done at Aaranya biosciences Pvt ltd Chennai.

3. Result and Discussion

3.1 Phytochemical Studies

The results of the phytochemical test Table 1 show the presence of various phytochemicals such as flavonoids, alkaloids, steroids, lipids, terpenoids, triterpenoids, phlobatannin. Phytochemicals are secondary metabolites which are produced and used by the plants for protection and repair process within the natural environment. Flavonoids are known to possess strong anticancer activity and are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage^{6,8}. Phenols, flavonoids and tannins are good antioxidant substances which have anti-diarrhoeal activity³ and prevent or control disorders related to oxidative stress⁹. Tannins are antiseptic in nature and can be used to increase the resistant body against parasites¹⁰. The presence of alkaloids has an antimicrobial activity². These phytochemicals possess specific physical, chemical and biological activities that make them useful as drugs.

Table 1. The phytochemical constituents of *Physalis minima* leaf and stem extract

Phytochemical constituents	Presence
Alkaloids	+
Phlobatannins	+
Triterpenoids	+
Flavonoids	+
Lipids	+
Steroids	+
Terpenoids	+

(+ present)

3.2 Antibacterial Assay

The antibacterial assay for chloroform extracts of leaf and stem were done against 4 bacterial strains namely *Enterococcus faecalis*, *Klebsiella oxytoco*, *Salmonella typhimurium* and *Lactobacillus acidophilus*. The antibacterial results showed minimal inhibitory effect against all strains. The inhibition zone was only found in *Klebsiella oxytoco* and the other strains showed resistance against the extract. The inhibition zone against *Klebsiella oxytoco* is: Ampicillin (positive control) – 1.5cm, Chloroform (negative control) – 1.0cm, Leaf extract – 3.0cm, Stem extract – 2.0cm. Through this method successful antimicrobial susceptibility test against the sample extract were done and the inhibition zone were found only for *Klebsiella oxytoco*.

3.3 FTIR Interpretation

The ground sample of leaf and stem of *Physalis minima* has been done with the Fourier Transform Infrared spectroscopy at 200 to 800 nm^{8,11} for which the following results have been obtained. The FTIR results confirmed the presence of aldehyde, phenol, alkanes, alkyls, amino acid, aromatic amino acids and secondary alcohol. On comparing both the extracts, similar functional groups were identified. The graphical representation of different functional group present is given in Figures 1 and 2. Each of them represents several peaks at different wavenumbers given at cm^{-1} at the far IR region³. Tables 2 and 3 represents the functional group present in the leaf and stem sample respectively by correlating it with the FTIR peaks corresponding wavenumbers.

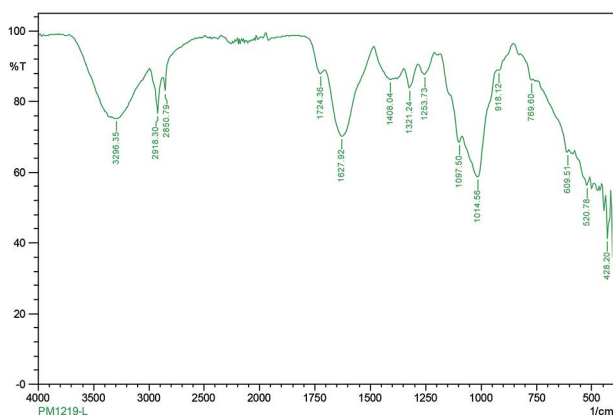


Figure 1. FTIR peak for leaf sample Transmittance% vs Wavenumber(1/cm).

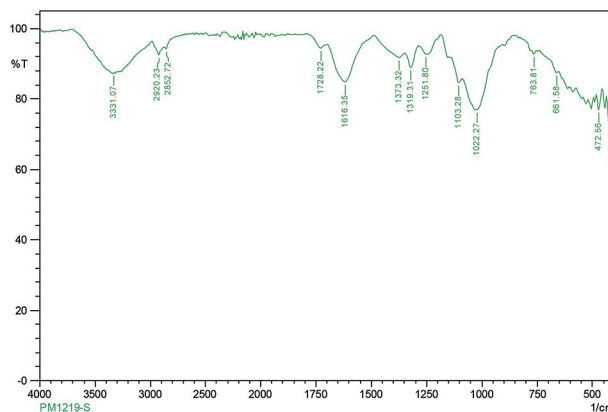


Figure 2. FTIR peak for stem sample Transmittance% vs Wavenumber(1/cm).

Table 2. FTIR peak value correspondence for leaf sample

Wave Number (cm-1)	Functional Group/ Assignment
3296.35	Normal polymeric O-H stretch/ O-H
2918.3	Methylene C-H asymmetric stretch/ CH ₂
2850.79	Methylene C-H symmetric stretch/ CH ₂
1724.36	Aldehyde/-CHO
1627.92	Alkenyl C=C stretch / C=C
1408.04	Phenol or tertiary alcohol bend/ -OH
1321.24	Phenol or tertiary alcohol bend/ -OH
1253.73	Aromatic ether (aryl-O-stretch)
1097.5	Aliphatic Fluoro group/ C-F stretch
1014.56	Cyclohexane ring vibration
918.12	Vinyl/ C-H
769.60	Methyne/ -CH
609.51	Aliphatic chloro compound/ C-Cl
520.78	Aliphatic iodo compound/ C-I
428.20	Aryl disulphide stretch/ S-S stretch

3.4 GC-MS

The GC-MS result showed the presence of bioactive compounds. Around 10 and 14 compounds were detected in leaf and stem extract of *Physalis minima* respectively. The active compounds, retention time, molecular formula, molecular weight, peak area and molecular structure are given in the Tables 4 and 5 for leaf and stem extract respectively. The most prevailing compounds in leaf extract are 3,7,11,15-tetramethyl-2-hexadecen-1-ol (18.935), Octadecanoic acid (19.675),

Table 3. FTIR peak value correspondence for stem sample



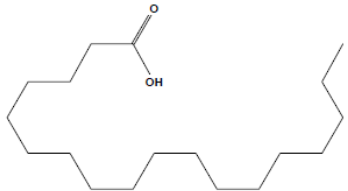


Wave Number (cm-1)	Functional Group/ Assignment
3331.07	Normal polymeric OH stretch
2920.23	Methylene C-H asymmetric/CH ₂
2852.72	Methylene C-H symmetric/ CH ₂
1728.22	Aldehyde/ -CHO
1616.35	Aromatic ring stretch/ C=C-Ca
1373.32	Gem- dimethyl or iso-doublet/-CH ₃
1319.31	Primary or secondary O-H in-plane bend
1251.8	Primary or secondary in-plane bend
1163.28	Aliphatic fluoro compound/ C-F stretch
1022.27	Organic siloxane or Silicone(Siliconeoxy-compound)/ So-O-Si
763.81	Monosubstitites(phenyl)/ -CH
661.58	Alkyne/ C-H bend
472.56	Polysuphide/S-S stretch




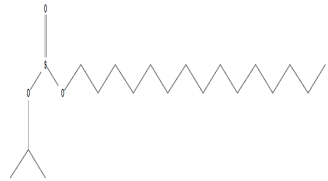
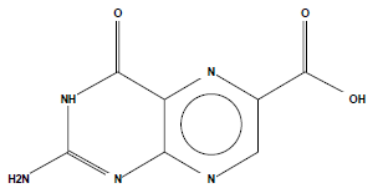
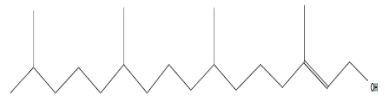

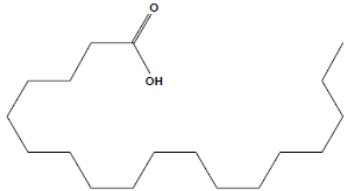


Heptacosane, 1-chloro (22.701), Heptacosane (25.312), Pterin-6-carboxylic acid (31.480) and in stem extract are N-hexadecanoic acid (21.596), 2-bromotetradecane (24.867), Hexatriacontane (26.618), Docosanoic acid, docosyl ester (27.198), Sulfurous acid, 2-propyl tetradecyl ester (28.594). Heptacosane, 1-chloro, Heptacosane, N-hexadecanoic acid and Hexatriacontane are present in both leaf and stem extracts. The Figures 3 and 4 show the chromatogram of the compounds detected in leaf and stem extract respectively.

3.5 Cytotoxicity Activity

The MTT assay done on the chloroform leaf and stem extract of *Physalis minima* showed the cytotoxicity in % which is given in the Tables 6 and 7 respectively. The study which was carried out in HL60 cell line showed a positive result. The cytotoxicity is being increased as the concentration of the extract is increased. Both the

Table 4. GC-MS result for leaf extract of *Physalis minima*

RT	Compound Name	Molecular Formula	Molecular Weight	Peak Area	Molecular Structure
18.935	3,7,11,15-tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	9.731	
19.335	Phytol	C ₂₀ H ₄₀ O	296	3.860	
19.675	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	18.398	
21.071	N-hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	5.793	
22.211	Hexatriacontane	C ₃₆ H ₇₄	506	4.476	

22.701	Heptacosane, 1-chloro	$C_{27}H_{55}Cl$	414	3.002	
24.707	Tetratriacontane	$C_{34}H_{70}$	478	8.396	
25.312	Heptacosane	$C_{27}H_{56}$	380	7.529	
27.743	Sulfurous acid, pentadecyl 2-propyl ester	$C_{18}H_{38}O_3S$	334	3.388	
31.480	Pterin-6-carboxylic acid	$C_7H_5O_3N_5$	207	9.298	
RT	Compound Name	Molecular Formula	Molecular Weight	Peak Area	Molecular Structure
18.935	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	9.731	
19.335	Phytol	$C_{20}H_{40}O$	296	3.860	
19.675	Octadecanoic acid	$C_{18}H_{36}O_2$	284	18.398	
21.071	N-hexadecanoic acid	$C_{16}H_{32}O_2$	256	5.793	
22.211	Hexatriacontane	$C_{36}H_{74}$	506	4.476	




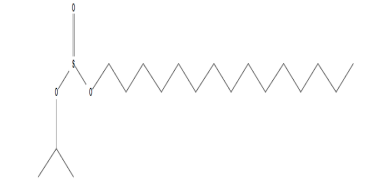
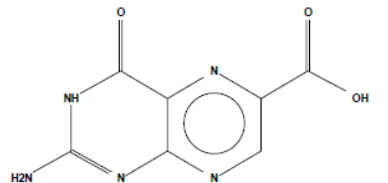

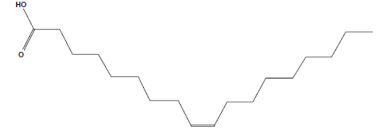
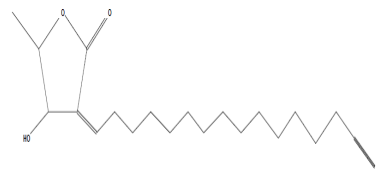
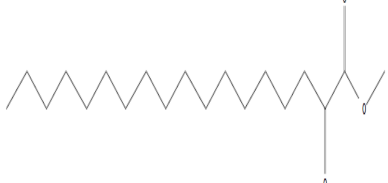

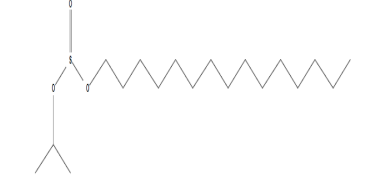



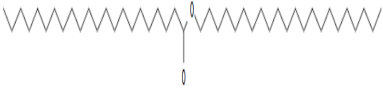


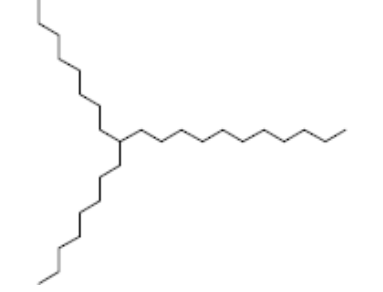

22.701	Heptacosane, 1-chloro	$C_{27}H_{55}Cl$	414	3.002	
24.707	Tetratriacontane	$C_{34}H_{70}$	478	8.396	
25.312	Heptacosane	$C_{27}H_{56}$	380	7.529	
27.743	Sulfurous acid, pentadecyl 2-propyl ester	$C_{18}H_{38}O_3S$	334	3.388	
31.480	Pterin-6-carboxylic acid	$C_7H_5O_3N_5$	207	9.298	

Table 5. GC-MS result for stem extract of *Physalis minima*

RT	Compound Name	Molecular Formula	Molecular Weight	Peak Area	Molecular Structure
21.596	N-hexadecanoic acid	$C_{16}H_{32}O_2$	256	8.901	
22.611	Oleic acid	$C_{18}H_{34}O_2$	282	2.032	
22.666	2(3H)-furanone, 3-(15-hexadecynylidene) dihydro-4-hydroxy-5-methyl-,	$C_{21}H_{34}O_3$	334	2.437	
22.891	Octadecanoic acid, 2-oxo-, methyl ester	$C_{19}H_{36}O_3$	312	5.168	

23.602	Heptacosane, 1-chloro	$C_{27}H_{55}Cl$	414	4.405	
24.247	Sulfurous acid, pentadecyl 2-propyl ester	$C_{18}H_{38}O_3S$	334	4.770	
24.867	2-bromotetradecane	$C_{14}H_{29}Br$	276	5.594	
25.467	Heptacosane	$C_{27}H_{56}$	380	5.794	
26.618	Hexatriacontane	$C_{36}H_{74}$	506	7.695	
27.198	Docosanoic acid, docosyl ester	$C_{44}H_{88}O_2$	648	9.703	
27.858	Behenyl chloride	$C_{22}H_{45}Cl$	344	6.971	
28.594	Sulfurous acid, 2-propyl tetradecyl ester	$C_{17}H_{36}O_3S$	320	9.598	
29.469	Eicosane, 9-octylpm1219s	$C_{28}H_{58}$	394	4.705	
30.469	Sulfurous acid, octadecyl 2-propyl ester	$C_{21}H_{44}O_3S$	376	4.678	

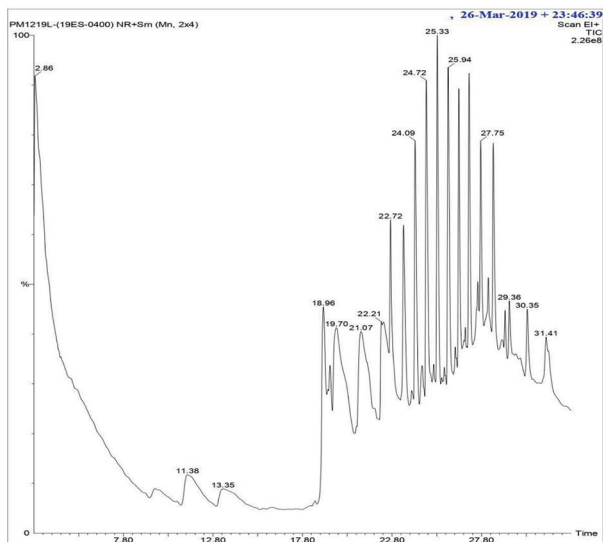


Figure 3. Chromatogram of compounds present in leaf extract of *Physalis minima*

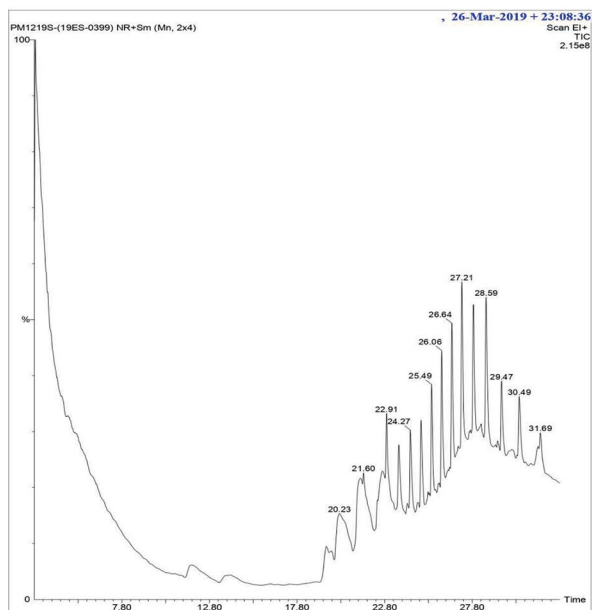


Figure 4. Chromatogram of compounds present in stem extract of *Physalis minima*.

stem and leaf extract have a good cytotoxicity effect on cell line. Comparing all the concentrations and their mean value, 250 $\mu\text{g/ml}$ concentration both the extracts showed the highest cytotoxic effect and 0.781 $\mu\text{g/ml}$ showed low activity. The leaf extract showed 79% whereas stem extract showed 72% as their maximum cytotoxic efficiency¹². Hence leaf extract showed a high activity against the cell line. The cytotoxicity graph plotted for leaf and stem extract is shown in Figures 5

and 7 respectively. The SEM images of the cytotoxicity tests are shown in Figures 6 and 8 respectively.

Table 6. Cytotoxic activity of leaf extract of *Physalis minima* on HL60 cell line

Concentration ($\mu\text{g/ml}$)	Cytotoxicity (%)	Cell Viability (%)
250	79.19	20.81
100	67.63	32.37
50	63.91	36.09
25	48.03	51.97
12.5	29.52	70.48
6.25	20.43	79.57
3.125	16.43	83.57
1.562	11.24	88.76
0.781	4.06	95.94

Table 7. Cytotoxic activity of stem extract of *Physalis minima* on HL60 cell line

Concentration ($\mu\text{g/ml}$)	Cytotoxicity (%)	Cell Viability (%)
250	71.9	28.1
100	59.5	40.5
50	33.2	66.8
25	22.8	72.8
12.5	16.2	83.8
6.25	12.56	87.44
3.125	9.91	90.09
1.562	4.22	95.78
0.781	2.01	97.99

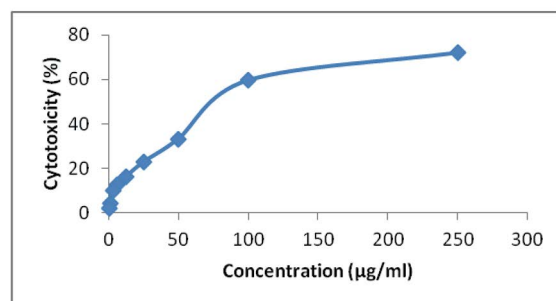


Figure 5. Graph showing the cell death percentage of leaf extract.

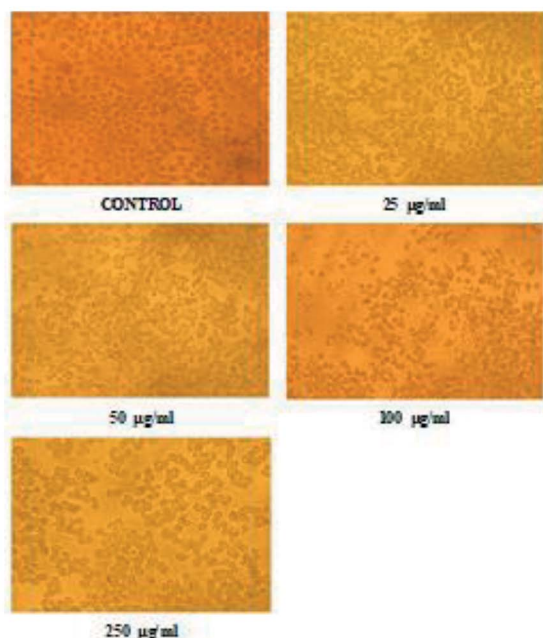


Figure 6. HL60 cell line reaction to different concentration of leaf extract.

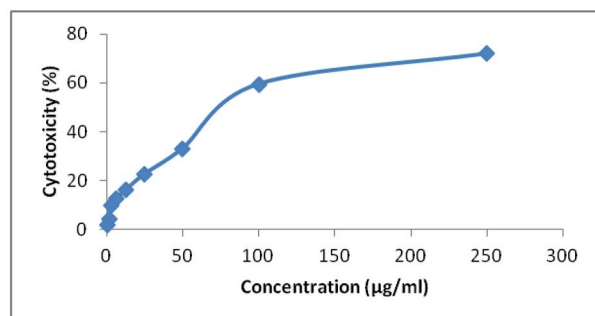


Figure 7. Graph showing the cell death percentage of stem extract.

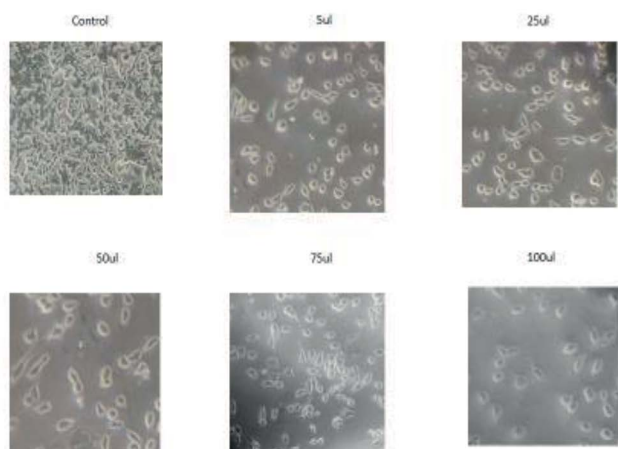


Figure 8. HL60 cell line reaction to different concentration of stem extract.

4. Conclusion

This study shows that the leaf extract of *Physalis minima* is rich in secondary metabolites and various bioactive compounds. It has a good cytotoxic activity in HL60 cell line. *Physalis minima* is a very common roadside plant which grows very well in South India, whose medicine values are not commercially well known. It has been used as a treatment for ulcer and gastroenteritis and also for indigestion in traditional medicine. Further knowledge about the plant will be useful in the discovery of various drugs. The compound can be isolated by further studies of this rarely known plant and it can be easily commercialized due to its ubiquitous presence.

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