WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 14, 1267-1278. Research Article ISSN 2277– 7105

EVALUATION OF BIOLOGICAL ACTIVITIES OF METHANOLIC EXTRACT OF PHYSALIS MINIMA LINN

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Article Received on
12 October 2021,

Revised on 02 Nov. 2021,
Accepted on 22 Nov. 2021

DOI: 10.20959/wjpr202114-22439

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ABSTRACT

Physalis species are used in folk medicine for phytotherapeutic properties from decades. The extracts of medicinal plants are known to possess antioxidant activity. In this study we carried out the methanolic extraction by soxhlet method and identified the phytoconstituents by UV and HPLC method of *Physalis minima* Linn. In this study we also investigated total phenolic content, total flavonoid content and antioxidant assay of methanolic extract of *Physalis minima* Linn. from the study it can conclude that *Phys. alis minima* Linn. having a promising antioxidant activity and it show the presence of flavonoid, phenolic compunds, withanolides, withaferine and B-sitisterol which is further investigated for their pharmacological activity and development of formulation.

KEYWORDS: Physalis minima Linn., Antioxidant, Withanolides, Withaferine.

INTRODUCTION

Physalis minima Linn. belongs to the family Solanaceae, which is commonly known as wild cape gooseberry in English. *Physalis minima* Linn. having various active phytoconstituents like phenols, flavonides, alkaloids, steroids and steroidal lactones with different pharmacological action like The plant is reported as antispasmodic, anti-diabetic, antioxidant, anti-inflammatory, antibacterial, diuretic, laxative, useful in inflammations, supplement for vit.c, enlargement of spleen and abdominal troubles, antilipid peroxidation, hypoglycemic, antitumor.

Experimental methods

1. Collection of Plant Material and Authentication

Whole fresh plant of *Physalis minima* Linn. was collected from farm of Wardha from the state of Maharashtra (India) and plant was authenticated by Dr. Prakash Itankar from the Department of Pharmacognosy, Pharmaceutical Sciences, R.T.M. Nagpur University, Nagpur.

2. Extraction of plant

The whole plant was shade dried and then grinds into coarse powder with the help of a suitable grinder. The dried plant material then extracted with methanol by Soxhlet extraction method for 72 hours. Then the semisolid extract freeze dried by adding cryo-protectant (5% mannitol) and store in amber colored glass bottle for further use.

3. Percent extract yield

The yield of dried extracts based on their dry weights was calculated using the following equation:

$\frac{W1}{W2}$ x 100

Where, W1= Weight of extract after solvent evaporation

W2= Weight of the dry plant material

4. Solubility studies

The solubility studies were carried out by dissolving the plant extract in different solvents such as methanol, ethanol, acetone, water etc.

5. Phytochemical screening

The preliminary phytochemical screening for phenols, quinones, flavonoids, alkaloids carbohydrates, proteins, amino acids, fixed oils, terpenoids, cardiac glycosides, steroids and tannins were carried out for identification of phytoconstituents present in methanolic extract of *Physalis minima* Linn. using standard procedure.

6. UV Spectrophotometric analysis of drug

Determination of **λmax in methanol**

i) Preparation of stock solution

The extract of *Physalis minima* Linn. (10mg) was weighed accurately and dissolved in 100 ml of methanol to prepare a stock solution of 100 ppm and scanned over range of 200-800 nm for determining λmax in methanol.

ii) Standard calibration curve

From the above prepared stock solution, 2-16 ml sample is withdrawn and diluted up to 10 ml to obtain 20-160 µg/mL of solution. A standard calibration curve was plotted against absorbance verses different concentrations at 216 nm.

7. HPLC analysis of herbal extract

The PM methanolic extract was analyzed by HPLC for the presence of phytoconstituents

Chromatographic conditions			
Compounds	**Withanolide A**	**Withaferine A**	**β-sitosterol**
Instrument	Agilent 1100 with DA detector	Agilent 1100 with DA detector	Agilent 1100 with DA detector
Column	C18 column (1.5 mm×4.6mm internal diameter, 5µm particle size)	C18 column (1.5 mm×4.6mminternal diameter, 5µm particle size)	C18 column (1.5 mm×4.6mminternal diameter, 5µm particle size)
Mobile phase	Methanol and 0.01 M ammonium acetate buffer (pH 5) (60:40)	Acetonitrile: Glacial acetic acid (6:4)	Acetonitrile:0.05% ortho phosphoric acid (90:10)
Flow rate	1.0 mL	1.0 mL	0.7 ml/min
Injection volume	20 µL	20 µL	20 µL
Wavelength	227 nm	227 nm	220 nm

8. Fourier transform infrared spectroscopy (FT-IR)

The FTIR spectrum of *Physalis minima* Linn. was determined by using Shimadzu IR Affinity-1S FTIR. The drug sample was triturated and mixed properly with potassium bromide in the ratio 1:100. The mixture was then put in to the sample holder of the FT-IR instrument and was scanned to obtain spectra in range from 4000 to 400 cm-1.

9. Differential scanning calorimetry (DSC)

The thermogram of *Physalis minima* Linn. was recorded with DSC. The thermal behavior was studied of sample in a hermetically sealed in the aluminium crucible and heated at a

constant rate of 10°C/minute over a temperature range of 40 to 400 ^{0}C. The sample was maintained under constant nitrogen gas flow.

10. X-ray diffractometry (XRD)

X-ray diffraction spectrum of *Physalis minima* Linn. was recorded using a powder x-ray diffractometer. The X-ray generator was operated at 40kV voltage and 40mA current using Cu-Kα as a source of radiation. The sample was measured in 2θ angle and range measured between 1-60°.

11. Total Phenolic Content (TPC)

Folin Ciocalteu's reagent method was used to determine total phenols present in PM. An aliquot (1ml) of extract was mixed thoroughly with 3 ml distilled water and then add 1 ml of Folin Ciocalteu's reagent(previously diluted with distilled water) added, followed by 2ml of Sodium bicarbonate (20%) was added to the mixture, mixed properly and incubated at dark for 30 min at room temperature in the dark place. Sample and standard absorbance was measured at 765 nm using UV spectrophotometer. A standard curve was plotted using different concentrations of gallic acid and the amount of total phenolic content was calculated as mg of gallic acid equivalents (GAE) per gram of dried extract.

12. Total Flavonoid Content (TFC)

The total flavonoid content was determined using the aluminium chloride colorimetric method with slight modification and quercetin used as a standard. The calibration curve was plotted with different concentrations of quercetin (20-100µg/ml). The 1 ml of extract (1000 µg/ml) was mixed with 0.1 ml of AlCl3 solution, 0.1 ml of 1M potassium acetate solution and 2.8 ml of distilled water. After 30 min of incubation at room temperature, the absorbance of sample and standard was measured at 415nm by using UV spectrophotometer. A solution containing all reagents except AlCl3which is replace by same amount of water used as blank. The result was expressed as mg of quercetin equivalents (QE) per gram of dried extract.

13. Antioxidant Assay by DPPH Scavenging method

In this method, solution of 0.1 mM DPPH was prepared in methanol and from this solution 1ml was mixed with 3 ml of extract solution prepared in methanol having 10, 20, 30, 40, 50 and 60 µg/ml of PM extract. The reaction mixture was swirled thoroughly. After 30 minutes of incubation, absorbance of the mixture was observed at 517 nm in UV-Visible spectrophotometer. L-ascorbic acid was taken as standard. The scavenging ability of sample

was estimated as per reported method. The IC50 values were determined using a linear regression equation. DPPH radical scavenging activity was expressed as the percentage inhibition calculated using the following equation;

% Inhibition =Ac−(Aa /Ac)/Ac X 100

where Ac and Aa represents the absorption of the control and extract respectively.

RESULTS AND DISCUSSION

Extraction and phytochemicals investigation

Extraction was successfully done by soxhlet apparatus method. Form all extracting solvent methanol can be selected because it show the presence of maximum constituents. Phytochemical screening revealed the presence of flavonoids, steroids, alkaloids, tannins and phenolic compounds as shown in table no 1.

Table no. 1: Phytoconstituents present in methanol extract of PM.

Tested groups	Methanolic extract of *Physalis minima* Linn.
Alkaloids	+
Flavonoids	+
Steroids	+
Tannin	+
Phenolic compounds	+
Terpenoids	+
Glycosides	-
Saponins	-

A. λ_{max} and standard calibration curve in Methanol

The λ_{max} of Physalis minima was found to be 216 nm **(Fig. 1)** and it behaved Beer-Lambert's law in concentration range of 20-160 µg/ml **(Fig. 2)**

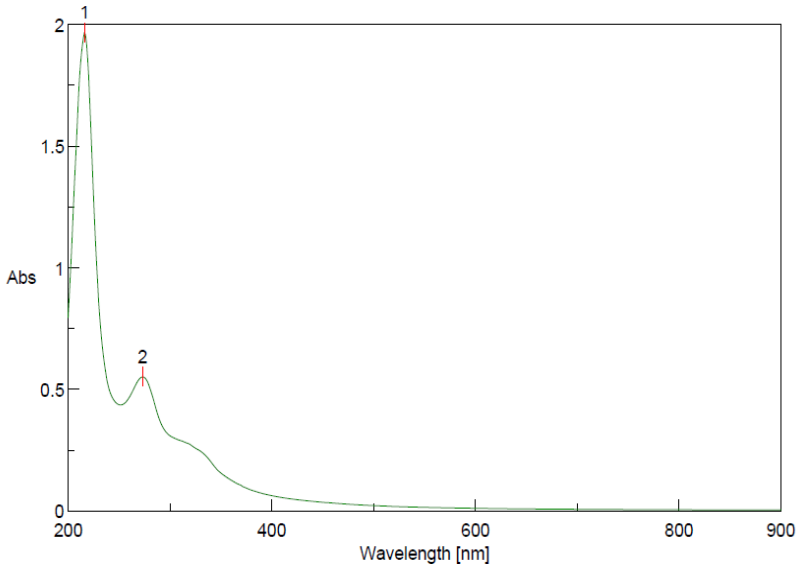

Figure no. 1: Spectrum of PM in Methanol.

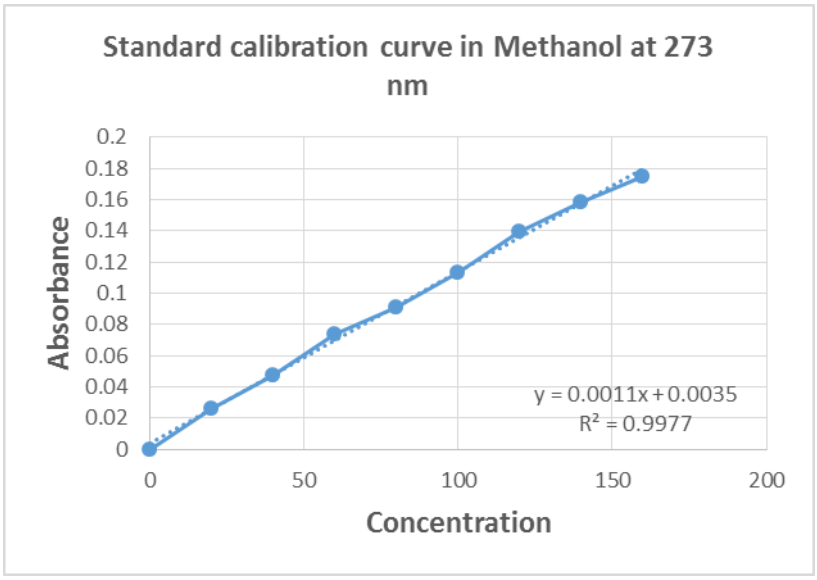

Figure no. 2: Standard calibration curve of PM in Methanol.

HPLC analysis of PM

The chromatograph obtained HPLC analysis revealed the presence of withanolide and withaferine in PM. The chromatograms of standard withanolideA, withaferine A and B-sitosterol are depicted in figure no. 4,5,6 with retention time 4.636, 3.596 and 5.0833. The chromatograph of PM depicted in figure no. 3 and shows the peaks with retention time 4.727, 3.640 and 5.029 due to presence withanolide, withaferine and β-sitosterol respectively.

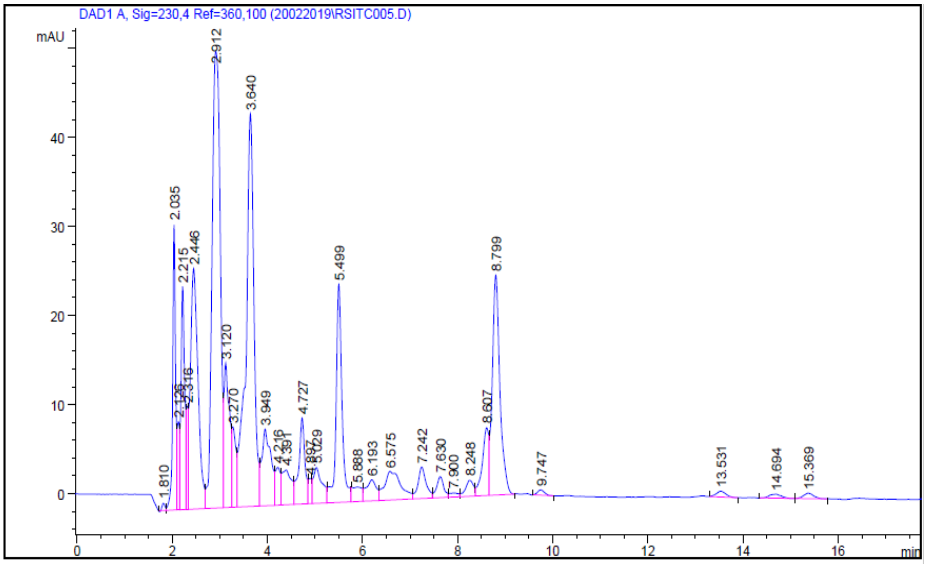

Figure no. 3: Chromatograph for PM.

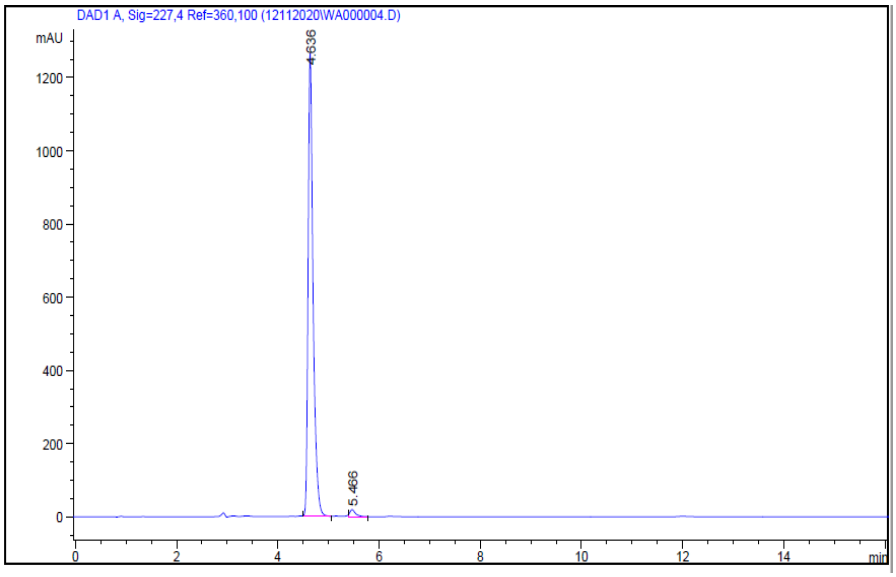

Figure no. 4: Chromatograph for withanolide.

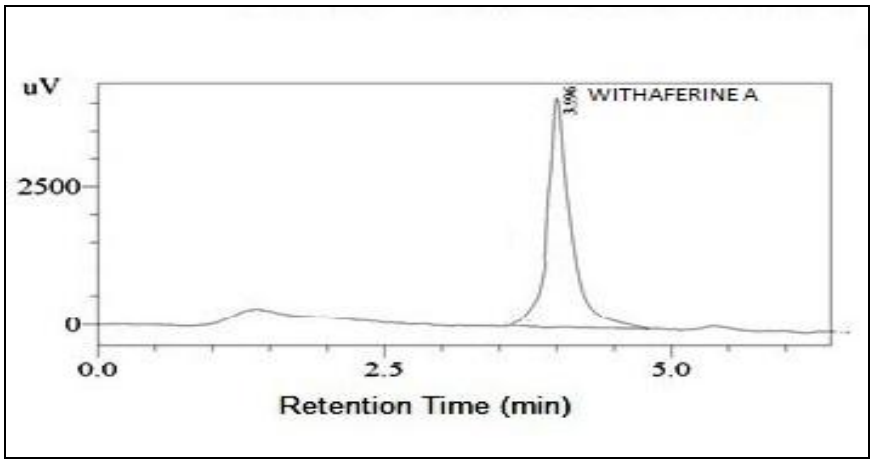

Figure no. 5: Chromatograph for withaferine A.

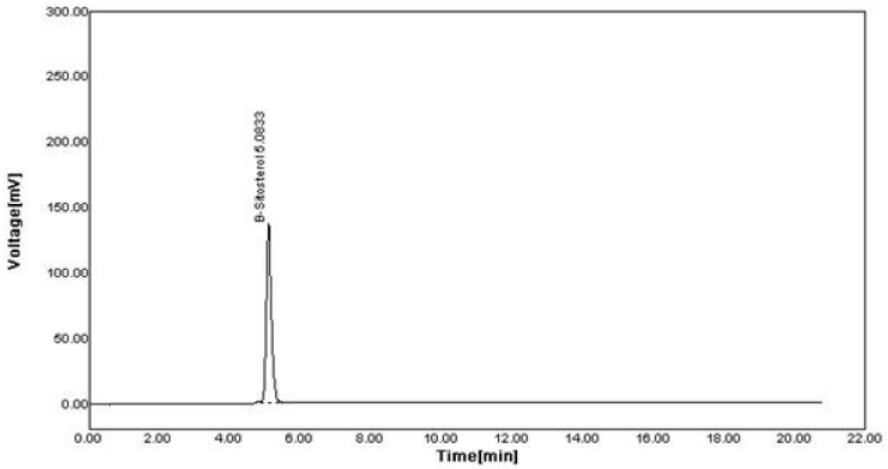

Figure no. 6: Chromatogram of B-sitosterol.

Fourier Transform Infrared Spectroscopy

The FTIR spectrum of PM (Fig. no.7) included the peaks at 3188.63 cm-1(O-H stretching), 2930.14(C-H stretching), 1612.21cm-1 (C=C stretching), 1073.23cm-1 (Carboxylic acid stretching), 1254.29 (C-O stretching) and 926.30 (C-H aromatic stretching).

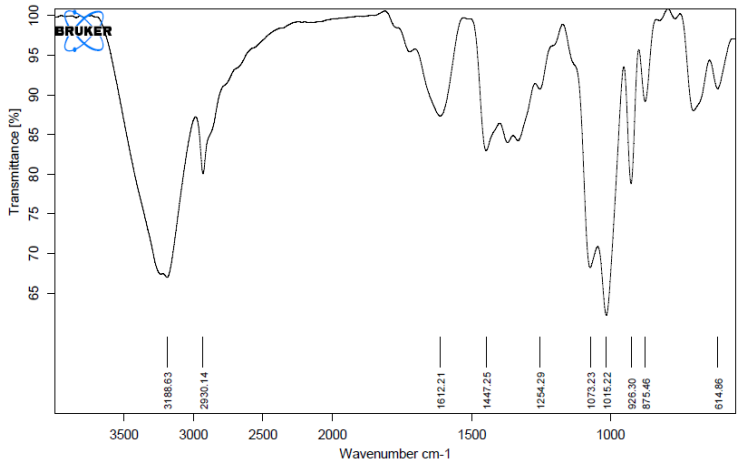

Figure no. 7: FT-IR spectrum of PM.

Differential scanning calorimetry (DSC)

DSC analysis was performed on PM. DSC thermogram of PM as shown in fig.no.8 revealed a broad endothermic peak at 93.66°C, 141.39°C

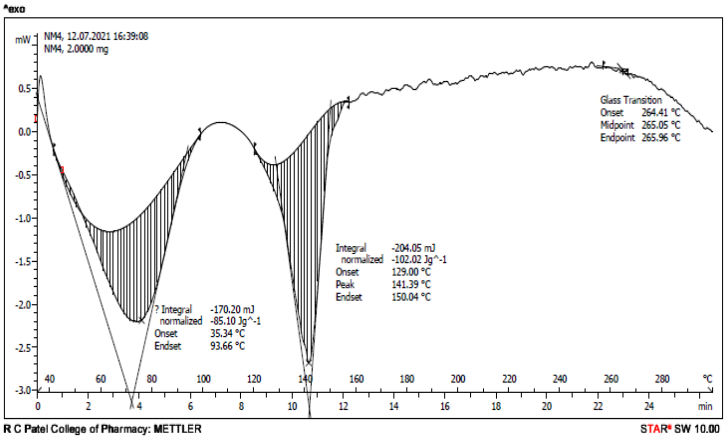

Figure no. 8: DSC thermogram of PM.

XRD (X-ray diffraction analysis)

The powder X-ray diffraction patterns of PM shown in fig.no.9. The peaks of PM showed sharp crystalline peaks that characterized with the crystallinity of an organic molecule.

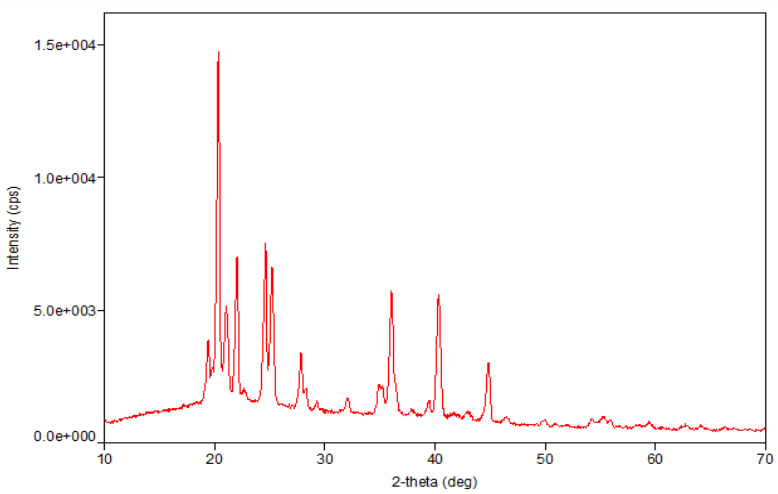

Figure no. 9: XRD spectrum of PM.

Determination of Total Phenolic Content (TPC)

The total phenolic content of methanolic extract of *Physalis minima* was done on the basis of a standard curve of gallic acid and linearity of the calibration curve was achieved between 2 to 10 µg/ml concentrations as shown in fig. no.10 for gallic acid. The regression coefficient (R2) was found to be 0.9922. The results showed that, the phenolic contents in the PM was found to be 52.44 mg GAE/g.

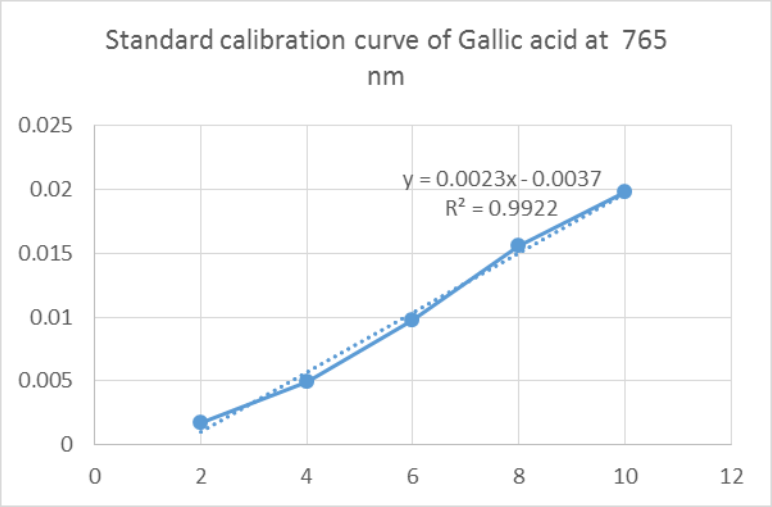

Figure no. 10: Standard calibration curve of Gallic acid.

Determination of total flavonoid content (TFC)

The total flavonoid content of methanolic extract of PM was done on the basis of the standard calibration curve of quercetin and the linearity of the curve is achieved between 20 to 100 µg/ml concentrations as shown in fig. no.11for quercetin. The regression coefficient (R2) was found to be 0.9882. The result showed that the flavonoid content in the PM was found to be 48.10 mg QE/g.

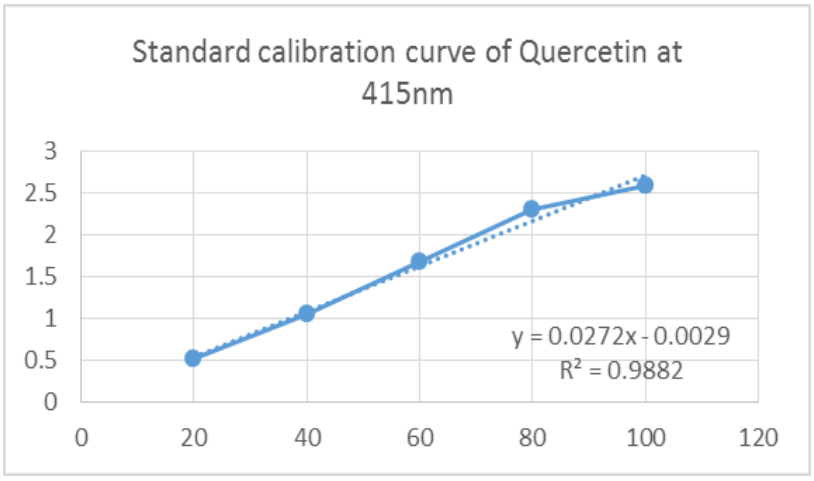

Figure no. 11: Standard calibration curve of quercetin.

Determination of antioxidant DPPH Scavenging Activity

Scavenging activity of PM is shown in fig. no.12. The activity was dose dependent and the maximum scavenging activity 82.66 was observed at 60 µg/ml concentration. The IC50 value was found to be 16.182±0.45 in the PM.

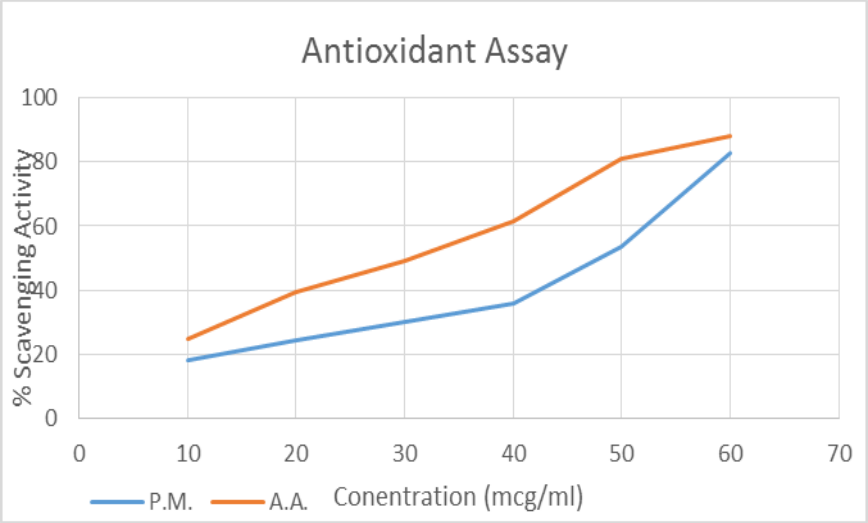

Figure no. 12: Antioxidant assay of PM and Ascorbic acid.

AKNOWLEDGEMENT

The authors gratefully acknowledge Department of Pharmaceutical Sciences RTM Nagpur University, Nagpur to provide all laboratory facilities for research work. We also thankful to Department of Pharmacognosy, RTM Nagpur University, Nagpur to identified the plant.

CONCLUSION

From the study it concluded that *Physalis minima* having a promising antioxidant activity and it show the presence of flavonoid, phenolic compunds, withanolides, withaferine and B-sitisterol which is further investigated for their pharmacological activity and development of formulation.

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