Research Article **ISSN: 2349 – 7106**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com

PHYTOCHEMICAL ANALYSIS AND *IN VITRO* HEPATOPROTECTIVE ACTIVITY OF EXTRACT OF *PSIDIUM CATTLEIANUM* SABINE LEAVES

Elamaran Tamil Jothi*[1], T. Savitha[1], C. K. Amritha[1], Joseph Paily[1]

[1*]Department of Pharmacology, Devaki Amma Memorial College of Pharmacy, Chelembra, Malappuram, Kerala, India.

ABSTRACT

The objective of the present study was to evaluate the phytoconstituents and *in vitro* hepatoprotective activity of extract of *Psidium cattleianum* Sabine leaves. The HPTLC profiling of plant extracts were carried out. The *in-vitro* hepatoprotective activity of hydroalcoholic extract of *P. cattleianum* (HAEPC) was screened by MTT Assay using Silymarin as standard. The HAEPC showed a significant hepatoprotective activity. The percentage cell viability was identified using MTT assay (*In-vitro*). The 50 Value of HAEPC was estimated. Results obtained suggests that the hydroalcoholic extract of *Psidium cattleianum* Sabine leaves exhibits a significant hepatoprotective activity.

KEYWORDS

Psidium cattleianum, Hepatoprotective activity, HPTLC and MTT assay.

Author for Correspondence:

Elamaran Tamil Jothi,

Department of Pharmacology,

Devaki Amma Memorial College of Pharmacy,

Malappuram, Kerala, India.

Email: tamilcologist@gamil.com

INTRODUCTON

The medicinal plants plays very important role in the health of human beings as well as animals. Herbal treatments are known as the most popular form of traditional medicine[1]. Plants which possess therapeutic properties or exert beneficial pharmacological effects on human body are designed as medicinal plants. They naturally synthesize and store Alkaloids, Sterols, Terpenes, Flavonoids, Saponins, Glycosides, Cyanogenics, Tannins, Resins, Lactones, Quinines, Volatile oils as secondary metabolites. Medicinal plants act as an effective source of traditional and modern medicine. People in different areas of the world use same or

similar plant or parts of plants for treating the same diseases[2].

Plants that possess therapeutic properties or exert beneficial pharmacological effects on the human body are generally designed as medicinal plants. Medicinal plants naturally synthesise and accumulate some secondary metabolite, like Alkaloids, Sterols, Terpenes, Flavonoids, Saponins, Glycosides, Cyanogenics, Tannins, Resins, Lactones, Quinines, Volatile oils etc. The medicinal plants have been used for treatment of illness and diseases. Researchers have found that people in different parts of the world tend to use the same or similar plants for treating the same illness[3].

MATERIAL AND METHODS

Collection and authentication

Fresh leaves of *Psidium cattleianum* Sabine was collected from Ambalavayal, Wayanad District, Kerala. The plant specimen (No.148219) was authenticated by Dr. A. K. Pradeep, Department of Botany, University of Calicut.

Extraction

Hexane, Chloroform and 70% Aqueous Ethanol extracts are prepared by successive solvent extraction of *Psdium cattleianum* Sabine leaves powder in Soxhlet apparatus. The filtrates obtained are distilled and concentrated under reduced pressure at low temperature and finally freeze dried and stored in a refrigerator until further use.

Phytochemical screening

Preliminary phytochemical analysis of the crude extract was carried out for qualitative estimation of phytoconstituents using different tests for Flavonoids, Phenols, Alkaloids, Tannins, Glycosides etc.

HPTLC profiling of plant extracts

The HPTLC profiling of plant extracts were carried out with solvent system Toluene: Ethyl acetate: Methanol (7:3:1). Equipment: CAMAG HPTLC system (Switzerland) with a sample applicator CAMAG ATS 4, Twin trough plate development chamber, Pre-coated silica gel plates Merck 60 F254 (0.2 mm thickness), CAMAG TLC SCANNER 3 with Deuterium lamp and win CATS software. Operation parameters: Band width: 6mm Application rate: 15µ/l Volume applied: 2ml Space between bands: 10mm Distance of plate side edge: 15mm Distance from the bottom of the plate: 10mm Distance of development: 80mm Development of Chromatogram:

The samples of n-Hexane, Chloroform and 70% ethanol extracts were spotted in the form of bands on pre-coated and pre-activated aluminium backed silica gel plates Merck 60 GF254 (10cm x 10cm width, 0.2mm thickness-E-Merk) by using a sample applicator CAMAG ATS 4. The plate was developed up to 80mm in ascending mode with solvent system, Toluene: Ethyl acetate: Methanol (7:3:1) at room temperature (28 ± 2°C) in a Twin Trough Chamber (Camag, Switzerland) which previously saturated with mobile phase. After development the air dried plate scanned at 254 nm and in 366 nm and in 550 nm (after spraying with anisaldehydesulphuric acid reagent followed by heating at 105 ^{0}C for 2 min) in CAMAG TLC SCANNER 3 using Deuterium lamp with win CATS software[4].

Development of Chromatogram

The samples of n-Hexane, Chloroform and 70% ethanol extracts were spotted in the form of bands on pre-coated and pre-activated aluminium backed silica gel plates Merck 60 GF254 (10cm x 10cm width, 0.2mm thickness-E-Merk) by using a sample applicator CAMAG ATS 4. The plate was developed up to 80mm in ascending mode with solvent system, Toluene: Ethyl acetate: Methanol (7:3:1) at room temperature (28 ± 2°C) in a Twin Trough Chamber (Camag, Switzerland) which previously saturated with mobile phase. After development the air dried plate scanned at 254 nm and in 366 nm and in 550 nm (after spraying with anisaldehydesulphuric acid reagent followed by heating at 105 ^{0}C for 2 min) in CAMAG TLC SCANNER 3 using Deuterium lamp with win CATS software[4].

Determination of Total Phenolic Content (TPC)

The total phenolic content of extract was determined by using Folin- Ciocalteu method. A standard gallic acid curve was constructed by preparing the concentrations ranging from 100-500µg/ml. Each of these dilutions were mixed 1 ml

of Folin-Ciocalteu reagent and allowed to stand for 3 minutes. Then 1ml of 20% sodium carbonate was added to the reaction mixture and adjusted to 10 ml with distilled water. The absorbance was recorded after 90 minutes at 765 nm spectrometrically. The same procedure was repeated with extract and total phenolic content was calculated. All the procedures were done in triplicate[5].

Determination of Total Flavonoid Content (TFC)

Aluminium chloride complex forming assay was used to determine the total flavonoid content of the extract. Quercetin was used as the standard at concentrations ranging from 100-500 µg/ml. Each of the quercetin dilution was mixed with 0.150 ml of 5% Sodium nitrate and allowed to stand for 6 minutes. Then 0.150 ml of 5% Aluminium chloride solution was added and allowed to stand for 5 minutes after which 0.5 ml solution of 1M Sodium hydroxide was added sequentially. The absorbance of this reaction mixture was recorded at 510 nm on UV spectrophotometer. The same procedure was repeated with extracts and total flavonoid content was calculated. All the procedures were performed in triplicate[5.]

In-vitro pharmacological evaluations

Hepatoprotective activity (MTT assay)

HepG2 (Human Hepatocellular Carcinoma) cell line was purchased from NCCS Pune was maintained in Dulbecco's modified eagles media from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's Modified Eagles medium(DMEM). The cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% CO_2 incubator. The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method. Cells seeding in 96 well plate: Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension (5x104 cells/well) was seeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% CO_2 incubator. Preparation of compound stock: 1mg of the Hydro alcoholic extract of *Psidium cattleianum* Sabine leaves was weighed and completely dissolved in 1mL DMEM using a cyclomixer. The extract solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility. Carbon tetrachloride (20mM) was added to induce toxicity. Cytotoxicity Evaluation: After attaining sufficient growth, Carbon tetrachloride (20mM) was added to induce toxicity and incubated for one hour, prepared extracts in 5% DMEM were five times serially diluted by two fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 500µl of 5% DMEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% CO_2 incubator.

Cytotoxicity Assay by Direct Microscopic observation: Entire plate was observed at an interval of each 24 hours; up to 72 hours in an inverted phase contrast tissue culture microscope and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Cytotoxicity Assay by MTT Method: Fifteen mg of MTT was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 3 0µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37ºC in a humidified 5% CO2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (DMSO was added) and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 540 nm[6].

RESULTS AND DISCUSSION

Preliminary phytochemical analysis

Phytochemical analysis of n-Hexane, chloroform and hydroalcoholic (70% ethanol) extracts of *P. cattleianum* Sabine leaves. The phytochemical screening of *P. cattleianum* Sabine leaves extracts possess the presence of various chemical constituents, among that the hydroalcoholic extract showed a positive result for all three tests for phenols and flavonoids. Phenolic compounds and flavonoids are the major targeting active constituents for hepatoprotective activity. So hydroalcoholic extract (HAEPC) was selected for further studies. It also possessed higher percentage yield in the extraction process.

HPTLC profiling of plant extract

All the three extracts of *P. cattleianum* leaves were subjected to HPTLC studies to identify the number and type of constituents present in it. Good resolution was obtained when Toluene: Ethyl acetate: Methanol solvent system used in the ratio of 7:3:1.

In this optimized solvent system, constitutes were clearly separated and the extracts showed varying number of spots/bands at different wavelengths. The n-hexane extract showed 7, 7, 12, chloroform extract showed 15, 14, 15 and hydroalcoholic extract showed 8, 8, 8 bands at 254nm, 366nm and 550nm respectively.

The R*f* value and percentage area of each band were calculated at different wavelengths for each extract. Maximum bands were observed at 550nm and their R*f* values and percentage peak areas were given in the table.

Total phenolic content estimation

The total phenolic content in the hydroalcoholic extract of *P. cattleianum* Sabine leaves (HAEPC) was estimated by Folin-Ciocalteu method taking gallic acid as standard. The total phenolic content present in the extract was found to be 41.45µg/ml.

Estimation of total flavonoid content the total flavonoid content in the hydroalcoholic extract of *P. cattleianum* Sabine leaves (HAEPC) was estimated by Aluminium chloride complex forming assay. The results are given in the following table: The total flavonoid content present in the extract was found to be as 32.50µg/ml.

In vitro hepatoprotective activity (MTT Assay)

Table No.10: Hepatoprotective activity of HAEPC against Carbon tetrachloride on HepG2 cell line. Carbon tetrachloride has a toxic effect on the cell lines and the percentage viability of the cells was about 43.39 ± 0.9620 %. HAEPC at a concentration of 100 µg/ml showed highest protection (83.10±0.5234 %) and showed least protection (47.39±0.6372 %) at a concentration of 6.25 µg/ml. The EC50 value of the extract in cell viability was found to as 8µg/ml. The extract produced a significant increase in protective effect on cell lines in a dose dependent manner. The morphological assessment of cell lines treated with Carbon tetrachloride indicate the presence of condensed nuclei, cell shrinkage and presence of apoptotic bodies. Cell lines showed a prominent effects after treatment with different concentration of HAEPC. The microscopic observations revealed that the extract has a protective effect on cell lines against carbon tetrachloride induced toxicity. At lower extract concentration, cell shrinkage and membrane blebbing was observed but decreased gradually on treatment with increasing concentrations of HAEPC. The EC50 was found to be 8µg/ml.

Hexane, Chloroform and 70% Aqueous Ethanol extracts are prepared by successive solvent extraction of *Psdium cattleianum* Sabine leaves powder in Soxhlet apparatus. The phytochemical screening of *P. cattleianum* Sabine leaves extracts possess the presence of various chemical constituents. Among that the Hydro alcoholic extract showed a positive result for all three tests for phenols and flavonoids. Phenolic compounds and flavonoids are the major targeting active constituents for hepatoprotective activity. It also possessed higher percentage yield in the extraction process.

The Preliminary phytochemical examination and quantitative estimation of bioactive components in hydroalcoholic extract of *Psidium cattleianum* Sabine leaves revealed the presence of various phytochemical constituents such as Glycosides, Saponins, Flavanoids, Phenolic compounds,

Tannins, Phytosterols and Triterpenoids, Amino acids and Proteins, Carbohydrates, Gum and mucilage, Vitamin C. The Total Phenolic and Flavonoid content were also estimated.

Hepatoprotective activity of HAEPC against Carbon tetrachloride on HepG2 cell line were performed (MTT Assay). The morphological assessment of cell lines treated with Carbon tetrachloride indicate the presence of condensed nuclei, cell shrinkage and presence of apoptotic bodies. Cell lines showed a prominent effects after treatment with different concentration of HAEPC. The microscopic observations revealed that the extract has a protective effect on cell lines against carbon tetrachloride induced toxicity.

At lower extract concentration, cell shrinkage and membrane blabbing was observed but decreased gradually on treatment with increasing concentrations of HAEPC. The IC50 of HAEPC was found to be 8µg/m.

Values are expressed as mean ± SEM. One way ANOVA comparison between negative group and control group and between negative group and treatment groups (Tukey's Method). The data are considered significant if *p<0.05, **p<0.01,***p< 0.001, ns-not significant.

Table No.1: Phytochemical Analysis

S.No	Phytochemical test	n-Hexane	Chloroform	70% Ethanol
Test for Alkaloids				
1	Mayer's test	—	—	—
2	Hager's test	—	—	—
3	Dragendroff's test	—	—	—
4	Wagner's test	—	—	—
Tests for Tannins and Phenolic compounds				
5	Ferric chloride test	—	+	+
6	Lead acetate test	—	—	+
7	Gelatin test	—	—	+
Test for Flavonoids				
8	Alkaline reagent test	—	+	+
9	Ammonium test	—	+	+
10	Lead acetate test	—	+	+
Tests for Triterpenoids				
11	Liebermann-Burchard's Test	+	+	—
12	Salkowski Test	+	+	+
Tests for Glycosides				
Test for Cardiac glycosides				
13	Baljet's test	—	+	+
14	Keller-Killani test	—	+	+
15	Legal's Test	—	—	—
16	Bromine water test	—	—	+
Tests for Anthraquinones glycosides				
17	Modified Borntrager's test	—	+	+

	Tests for Saponins			
1	Foam test	—	—	+
2	Olive oil test	—	+	+

Tests for Proteins and Amino acids				
3	Biuret test	+	+	+
4	Ninhydrin test	—	+	+
5	Xanthoprotein test	—	—	+
Test for Fats and Fixed oils				
6	Filter paper test	+	+	—
7	Saponification test	+	—	—
8	Coppers sulphate test	+	—	—
Tests for Carbohydrates				
9	Molisch's test	—	+	+
10	Fehling's test	—	—	+
11	Benedict's test	—	—	+
Tests for Gum and Mucilages				
12	Swelling test	—	+	+
Test for Vitamin C				
13	Sodium nitroprusside test	—	—	+
14	Sodium bicarbonate Test	—	—	+

Table No.2: Total flavonoid content estimation

S.No	Concentration(µg/ml)	Absorbance at 415 nm Mean± SEM
1	10	0.1166±0.0034
2	20	0.1701±0.0060
3	30	0.3118±0.0056
4	40	0.4603±0.0013
5	50	0.5952±0.0035
6	1 mg/ml of extract	0.3524±0.0032

Table No.3: Total Phenolic content

S.No	Concentration (µg/ml)	Absorbance at 750 nm Mean± SEM
1	10	2.1812±0.0034
2	20	2.4034±0.0061
3	30	2.6841±0.0147
4	40	2.9902±0.0100
5	50	3.2083±0.0048
6	1 mg/ml of extract	2.9961±0.0545

Table No.4: Hepatoprotective activity of HAEPC against Carbon tetrachloride on HepG2 cell line

S.No	Sample Concentration (µg/ml)	Percentage Viability (%) ± SEM
1	Control	100±0.0112
2	CCl4	43.39±0.9620***
HAEPC		
3	6.25	47.39±0.6372*
4	12.5	56.03±0.4600***
5	25	67.43±1.538***
6	50	68.69±0.4242***
7	100	83.10±0.5234***
IC50 of HAEPC = 8 µg/ml		

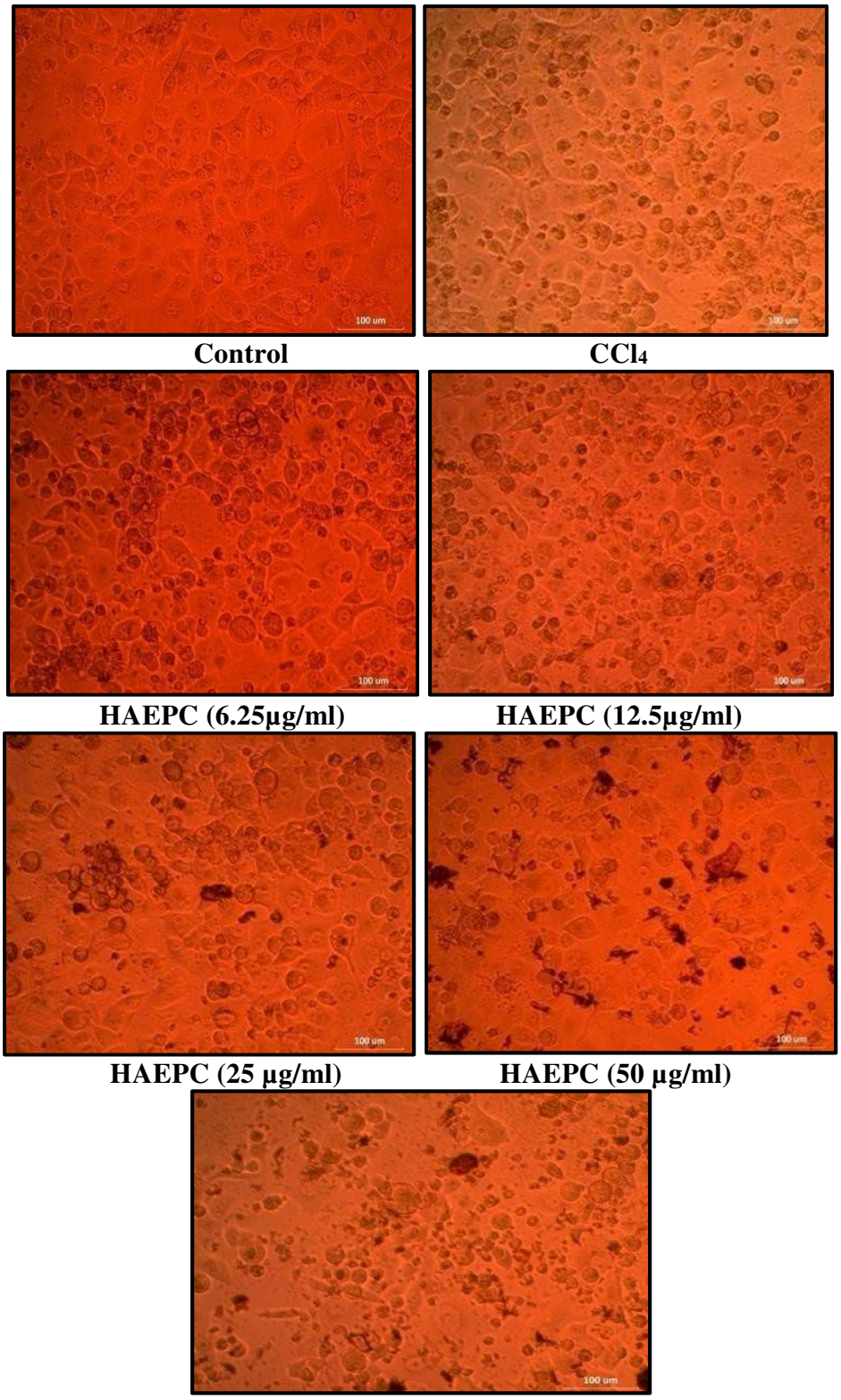

Figure No.1: Cell viability of Control and CCl4 induced on HepG2 cell lines by MTT assay. Cell viability of HAEPC on HepG2 cell lines by MTT assay

CONCLUSION

The study revealed that the Hydro alcoholic extract of *Psidium cattleianum* Sabine leaves was better choice for hepatoprotective activity. Thus it can be concluded that HAEPC possess significant hepatoprotective potential. Phytochemical constituents such as Flavonoids, Glycosides, Saponins, Phenolic compounds, Tannins, Triterpenoids, Amino acids and Proteins, Carbohydrates, Gum and mucilage and Vitamin C. The Further studies required to conclude the mechanism of action of the extract.

ACKNOWLEDGEMENT

I'm very thankful to Department of pharmacology, Faculty of Devaki Amma Memorial College of Pharmacy, Malappuaram, Kerala, India. I would like to thank the management, for providing necessary facilities to carry out this project.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBILIOGRAPHY

1. Kingshuk L, Swarnamoni D. Hepatoprotective activity of Ocimum sanctum alcoholic leaf extract against paracetamol-induced liver damage in Albino rats, *Pharmacognosy Res,* 3(1), 2011, 13-18.
2. Health topics A to Z. *Traditional medicine,* [www. allcountries.com]. 2016. http://www.allcountries.org/health/traditional_medicine.html.
3. Mohammad A M. Selected medicinal plants of Chittagong hill tracts, *International Union for Conservation of Nature,* 1(1), 2011, 1-4.
4. Murugesan S and Bhuvaneswari S. HPTLC fingerprint profile of methanol extract of the marine red alga Portieriahornemannii (Lyngbye) (Silva), *International Journal of Advances in Pharmaceutics,* 5(3), 2016, 61-65.
5. Shoib Baba A, Shahid Malik A. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of Arisaemajacquemontii Blume, *Journal of Taibah University for Science,* 9(4), 2015, 449-454.
6. Vishnu Kumar K S, Palaksha M N, Venkatesh K, Sandipkumar Y and Nayak R R. Antioxidant and hepatoprotective effect of methanolic extract of Origanummajorana in CCl4 induced liver injury in rats, *International journal of pharmacognosy,* 1(2), 2014, 144-152.

Please cite this article in press as: Elamaran Tamil Jothi *et al*. Phytochemical analysis and *in vitro* hepatoprotective activity of extract of *psidium cattleianum* sabine leaves, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences,* 7(2), 2019, 514-521.