Journal of Young Pharmacists, 2025; 17(3):571-580.
https://www.jyoungpharm.org

Original Article

Biological Profiling and Phytochemical Analysis of *Peristrophe bicalyculata*: Evaluating its Therapeutic Potential and Active Compounds

Raman Lakshmisundaram[1,*], Kalaivani Madhavaram Kuppusamy[2], Logeshwari Muruganandham[3], Krishna Priya Rajaram Baskaran[3], Dhanalakshmi Muniyandi[3], Mudiganti Ram Krishna Rao[4], Jayasutha Jayram[5,*]

[1]Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai, Tamil Nadu, INDIA.
[2]Department of Biomedical Sciences, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai, Tamil Nadu, INDIA.
[3]Faculty of Clinical Research, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai, Tamil Nadu, INDIA.
[4]Department of Industrial Biotechnology, Bharath Institute of Higher Education and Research, Selaiyur, Chennai, Tamil Nadu, INDIA.
[5]Department of Pharmacy Practice, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (DU), Porur, Chennai, Tamil Nadu, INDIA.

ABSTRACT

Background: The present study deals the biological property of a medicinal plant, *Peristrophe bicalyculata*. This is a common herb used for a number of ailments ethno-pharmacologically. In the present study, the antioxidant, anti-inflammatory and anti-cancer activity of the ethyl acetate extract of *P. bicalyculata* was reported. **Materials and Methods:** After collecting the plants from nearby hills of Chengalpattu, Tamil Nadu, the aerial part was washed and dried. The ethyl acetate extract of the plant was prepared using maceration method studied for its anti-oxidant, anti-inflammatory and cytotoxic activities. **Results:** Results revealed that the extract can able to scavenge the radicles at the IC_{50} value of 33.96 µg/mL. Similarly, the anti-inflammatory activity also revealed that IC_{50} value of protein denaturation assay and HRBC membrane stabilization assay was 125 µg/mL and 41.5 µg/mL respectively. In lung cancer cell line A549, 62.57% of the cell death was observed at 1000 µg/mL. Ao/EtBr staining reveals cell death is mainly due to apoptosis and necrosis was less observed. **Conclusion:** The study concludes that the plant has potential anticancer activity. Further studies on the bioactive compound behind the activity and their mechanism will provide a clear insight.

Keywords: *Peristrophe bicalyculata*, Antioxidant, Anti-Inflammatory, Anticancer, Molecular Docking.

Correspondence:
Dr. Raman Lakshmisundaram
Associate Professor (Research), Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai-600116, Tamil Nadu, INDIA.
Email: sundaram@sriramachandra.edu.in

Dr. Jayasutha Jayram,
Assistant Professor, Department of Pharmacy Practice, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (DU), Porur, Chennai- 600 116, Tamil Nadu, INDIA.
Email:jayasuthaj@sriramachandra.edu.in

Received: 09-04-2025;
Revised: 18-06-2025;
Accepted: 26-08-2025.

INTRODUCTION

Medicinal plants are used from ancient times in treating many ailments. A medicinal plant possesses many chemicals and essential oils which show different therapeutic properties. This therapeutic property has to scientifically evaluated to commercialize the medical property of as a drug (Sofowara *et al*., 2013). Pharmaceutical companies are glancing in to the medicinal plants for the interesting compounds with potent therapeutic activity and less side effects. WHO stated, 80% of the population in the universe are trusted on the medicinal plants to cure many ailments before visiting the physicians (Aye *et al*., 2019). Searching for drug from medicinal plant should be carried out to develop a useful drug which might be from unexplored plants also. Therefore, many labs are focusing on the primary screening of the therapeutic property from the crude extract of the medicinal plant which helps to develop the potent drug in treating disease (Farnsworth *et al*., 1985). In the present study one such medicinal plant *Peristrophe bicalyculata* is chosen to explore the antioxidant, anti-inflammatory and anti-cancer activity in the crude extract.

Peristrophe bicalyculata (synonym *Dicliptera paniculata*, (Forssk.) I. Darbysh) is a common herb used for a number of ailments ethno-pharmacologically. This plant is also called as Goddess of Mercy This plant is widely disseminated from tropical Africa to India (Abdulazeez *et al*., 2022). The herb has expectorant, analgesic, anti-inflammatory, antipyretic, antibacterial, anti-hypertensive and anti-cancer properties. It is also used to cure snake poisoning, bone fracture, sprain, fever, cold and for

DOI: 10.5530/jyp.20250031

ear and eye ailments (Reshmi *et al.*, 2010). The anti-bacterial properties of this plant were studied against various pathogens affecting the respiratory tract and chloroform extract showed best antimicrobial activity than the other extract (Arya, 2018). The ameliorative property against the parasitic disease of *P. bicalyculata* was studied in the rats infected by *Trypanosoma brucei* (Abimbola *et al.*, 2013). Ogunwande *et al.*, 2010 also have reported the essential oil constituents and their biological roles of this plant (Ogunwande *et al.*, 2010). In the present study, the antioxidant, anti-inflammatory and anti-cancer activity of the ethyl acetate extract of *P. bicalyculata* was reported.

MATERIALS AND METHODS

Extraction of plant

Collection of *Peristrophe bicalyculata* plant was carried out from the hilltop at Chengalpattu, Tamil Nadu. After getting authentication from the Botanist the aerial parts of the plant were separated and shade dried. Using the maceration procedure, ethyl acetate extract was prepared. After 48 hr, extract was evaporated and the dried extract was used for further analysis.

Antioxidant studies

ABTS Radical Scavenging activity

7.4 mM of ABTS was incubated with 2.6 mM of Potassium persulfate was incubated for 16 hr which helps in the generation of free radical 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+). The absorbance was measured at 734 nm after incubating 16 hr. Dilute the samples with methanol, until the absorbance reaches 0.706±0.001 at 734 nm. After the dilution process, 3.9 mL of ABTS•+ was mixed with 100 µL of the plant extract at different concentrations (25, 50, 100, 250, 500 and 1000 µg/mL). Sharply after 6 min, the absorbance was measured at 734 nm (Vijayamuthuramalingam *et al.*, 2017). The percentage of inhibition was calculated using the formula

% of inhibition=(Abs of control-Abs of test) /Abs of control x100

To compare the results with the standard, Ascorbic acid was used.

Reducing power capacity

This experiment is based on the property to reduce ferric (III) to ferrous (II) by the compounds present in the plant extract (Vijayamuthuramalingam *et al.*, 2017). Various concentration of the samples from 25-1000 µg/mL of 1 mL was mixed with 2.5 mL of phosphate buffer (0.2 M, pH=6.6) and 2.5 mL of 1% potassium ferricyanide were added and incubated at 50°C for 20 min. To stop the reaction after the incubation, 2.5 mL of 10% TCA was added. Equal volume of the distilled water was added to dilute the samples. Followed this 0.1% $FeCl_3$ was added and allowed to stand for 10 min. Optical density was measured at 700 nm. Vitamin C was used as the standard.

Anti-inflammatory studies

Inhibition of Protein Denaturation

Following homogenization with 1 mL of an aqueous solution of Bovine Serum Albumin (BSA) (5%), various concentrations (100, 200, 500, and 1000 µg/mL) of plant extracts or diclofenac sodium was incubated at 27°C for 15 min. The control tube was made up of distilled water and BSA. Protein denaturation was achieved by subjecting the mixture to a 10-min water bath at 70°C. The mixture was maintained at room temperature, and each mixture's activity was measured at 660 nm (Djuichou *et al.*, 2019). Every test was administered thrice. The inhibition % was computed using the following formula:

% of inhibition=(Abs of control -Abs of test) /Abs of control x100

HRBC membrane stabilization assay

An equal volume of sterilised Alsiever medium (2% (w/v) dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% sodium chloride in water) was combined with the drawn blood. After centrifuging the blood again for 10 minutes at 3000 rpm, the packed cells were cleaned with saline (pH 7.2, NaCl 0.9%), and then 10% (v/v) solution was prepared using saline. The plant extract, 1 mL phosphate buffer (0.15 M, pH 7.4), 2 mL saline (0.36%), and 0.5 mL HRBC solution were all included in the assay combination. Diclofenac served as the benchmark medication. The control was 2 mL of distilled water rather than hyposaline. The test concoctions were centrifuged at 3000 rpm for 10 min after incubated at 37°C for 30 min (Parameswari *et al.*, 2019). Optical Density was measured at 560 nm and the hemolysis % was calculated using the equation:

Hemolysis (%)=(Optical density of test sample/Optical density of control)×100

Anticancer activity of the extract

MTT assay

To study the anticancer activity, A549 lung cancer cell line was used. The cells were maintained in the Co2 incubator at 37°C. The healthy cells were seeded in 96 well plate at 10^3 cell density. Once the cells reached the confluency, various concentration of extract was treated to the cells. After the 24 hr of incubation period, the plates were taken and removed the media and washed with PBS. The cells were treated with 20 µL of MTT and incubated further for 2 hr. After the incubation period, the formed formazon crystals were dissolved with DMSO and measured the absorbance at 570 nm

Ao/EtBr staining

The cells were grown in the 6 well plate with the density of $5x10^5$ cells per well and incubated. Once confluent, the cells were

treated with the extract at the concentration of 500 and 1000 µg/mL. After 24 hr of incubation period, media was removed, washed with PBS and then 10 µl of 1 mg/mL concentration of Acridine Orange (AO) and Ethidium Bromide (EtBr) was added in the six well plate containing PBS. The samples were visualized using flouresence microscope in triple filter and observed for the apoptotic and necrotic changes.

RESULTS

Yield of extraction

The cold maceration of the plant powder with ethyl acetate solvent was dehydrated and the percentage of yield was calculated as 6.2%.

Study on Antioxidant activity of the plant

ABTS radical scavenging assay

Two techniques were used to assess the plant's antioxidant activity namely the reducing power activity and the ABTS radical scavenging assay. The extract improved the free radical scavenging activity of ABTS* in a dose-dependent manner. 33.96 µg/mL is the extract's IC_{50} value for scavenging the ABTS radical (Figure 1a).

Reducing Power assay

Reducing capability of the extract was studied on how the compounds present in the extract have the capability to reduce the Fe^{3+} (ferricyanide complex) and form the Fe^{2+} (ferrous compound). The extract showed potent reducing power activity with the absorbance of 0.733±0.0001 at 1 mg/mL. The standard ascorbic acid showed absorbance of 0.91±0.0004 at 1 mg/mL (Figure 1b).

Table 1: GC MS profile of Peristrophe bicalyculata.

Ret. Time	Name of the Compound	Mol. Mass	Mol. Formula	% Peak area	Possible medicinal Properties
4.08	Benzene, 1-ethynyl-4-fluoro-	120	C_8H_5F	13.78	Not known
4.18	Pyrrolidine, 2-butyl-1-methyl-	141.2	$C_9H_{19}N$	29.45	Catechol-O-Methyl-Transferase-Inhibitor, Catechol-O-Methyltransferase-Inhibitor, Methyl-Donor, Methyl-Guanidine-Inhibitor
5.18	trans-Traumatic acid	228.1	$C_{12}H_2OO_4$	0.34	Catechol-O-Methyl-Transferase-Inhibitor, Decreases Glutamate Oxaloacetate Transaminase, Decreases Glutamate Pyruvate Transaminase, Glucosyl-Transferase-Inhibitor, Glutathione-S-Transferase-Inhibitor, Increase Glutathione-S-Transferase (GST) Activity, Increases Glyoxalate Transamination, Reverse-Transcriptase-Inhibitor, Transdermal, Acidifier, Acidulant, Arachidonic acid-Inhibitor, Arachidonic-Acid-Inhibitor, Increases Aromatic Amino Acid Decarboxylase Activity
5.30	.alpha.-D-Glucopyranoside,O-.alpha.-D-glucopyranosyl-(1.fwdarw.3)-.beta.-D-fructofuranosyl	504.2	$C_{18}H_{32}O_{16}$	10.76	5-Alpha-Reductase-Inhibitor, Alpha-Agonist, Alpha-Amylase-Inhibitor, Alpha-Glucosidase-Inhibitor, Alpha-Reductase-Inhibitor, HIF-1alpha-Inhibitor, IkappaB-alph a-Phosphorylation-Inhibitor, Increase Alpha-Activity, Interleukin-1-alpMannosidase ha-Inhibitor, Testosterone-5-Alpha-Reductase-Inhibitor, TNF-alpha-Inhibitor, Aldehyde-Oxidase-Inhibitor, Anticancer, Antidote, Antiretinitic, Antitumor, Catechol-O-Methyl-Transferase-Inhibitor, Catechol-O-Methyltransferase-Inhibitor, Increases Osteocalcin

Ret. Time	Name of the Compound	Mol. Mass	Mol. Formula	% Peak area	Possible medicinal Properties
5.56	[1-(3,3-Dimethyloxiran-2-ylmethyl)-3,7-dimethylocta-2,6-dienyl]trimethylsilane	294.2	$C_{18}H_{34}OSi$	0.52	NK
5.98	1,2,3,5-Cyclohexanetetrol, (1.alpha.,2.beta.,3.alpha.,5.beta.)-	148.1	$C_6H_{12}O_4$	22.70	NKs
6.12	l-Gala-l-ido-octonic lactone	238.1	$C_8H_{14}O_8$	2.15	Beta-Galactosidase-Inhibitor, Galactagogue, 12-Lipoxygenase-Inhibitor, 5-Lipoxygenase-Inhibitor, Anti-LDL, Anticancer, Anticarcinomic, AntiCorpus-Luteum, Antidote, Antitumor, Benzodiazepine-Receptor Ligand
6.14	cis-10-Heptadecenoic acid	268.2	$C_{17}H_{32}O_2$	2.50	Acidifier, Acidulant, Arachidonic acid-Inhibitor, Increase Aromatic Amino Acid Decarboxylase Activity, Inhibits Production of Uric Acid,
6.29	trans-13-Octadecenoic acid	282.3	$C_{18}H_{34}O_2$	5.83	Catechol-O-Methyl-Transferase-Inhibitor, Decreases Glutamate Oxaloacetate Transaminase, Decreases Glutamate Pyruvate Transaminase, Glucosyl-Transferase-Inhibitor, Glutathione-S-Transferase-Inhibitor, Increase Glutathione-S-Transferase (GST) Activity, Increases Glyoxalate Transamination, Reverse-Transcriptase-Inhibitor, Transdermal, Acidifier, Acidulant, Arachidonic acid-Inhibitor, Increases Aromatic Amino Acid Decarboxylase Activity, Inhibit Production of Uric Acid
6.34	12-Hydroxy-14-methyl-oxa -cyclotetradec-6-en-2-one	240.2	$C_{14}H_{24}O_3$	1.15	Decrease Glutamate Oxaloacetate Transaminase, Decreases Oxalate Excretion, Low Oxalate, Decreases Endothilial Leukocyte Adhesion, Decreases Endothilial Platelet Adhesion, Encephalopathic, Endocrinprotective, Endorphinogenic, Endothelium-Dependent, Endothelium-Derived Relaxing Factor Promoter, Energizer, Enterocontractant
6.66	D-Mannoheptadecane-1,2,3,4,5-pentaol	320.3	$C_{17}H_{36}O_5$	0.34	'Smart-Drug', 17-beta-hydroxysteroid dehydrogenase-Inhibitor, Alcohol-Dehydrogenase-Inhibitor, Anticancer , Antidote Antileukotriene-D4, Circulatory-Depressant, CNS-Depressant, Coronary-Dilator, Cyclin-D1-Inhibitor, , Decalcifier, Decarboxylase-Inhibitor, Decongestant, Decreases C-Teleopeptide Excretion, Decrease Deoxypyridinoline Excretion, Decreases Endothilial Leukocyte Adhesion, Decreases Endothilial Platelet Adhesion
6.84	D-chiro-Inositol, 3-O-(2-amino-4-((carboxyiminomethyl) amino)- 2,3,4,6-tetradeoxy-. alpha.-D-arabino-hexopyranosyl)-	379.2	$C_{14}H_{25}N_3O_9$	2.26	NK

Ret. Time	Name of the Compound	Mol. Mass	Mol. Formula	% Peak area	Possible medicinal Properties
6.87	1,2,3,4,5-Cyclopentanepentol	150.1	$C_5H_{10}O_5$	0.65	NK
6.94	Octanoic acid, 2-methyl-	158.1	$C_9H_{18}O_2$	5.29	Catechol-O-Methyl-Transferase-Inhibitor, Catechol-O-Methyltransferase-Inhibitor, Methyl-Donor, Methyl-Guanidine-Inhibitor, Acidifier, Acidulant, Arachidonic acid-Inhibitor, Arachidonic-Acid-Inhibitor, Increase Aromatic Amino Acid Decarboxylase Activity. Inhibit Production of Uric Acid, Urinary-Acidulant, Urine-Acidifier
7.15	11-Bromoundecanoic acid	264.1	$C_{11}H_{21}BrO_2$	0.78	Acidifier, Acidulant, Arachidonic acid-Inhibitor, Arachidonic-Acid-Inhibitor, Increase Aromatic Amino Acid Decarboxylase Activity, Inhibit Production of Uric Acid, Urinary-Acidulant, Urine-Acidifier
8.15	Methyl stearate	298.3	$C_{19}H_{38}O_2$	3.80	Catechol-O-Methyl-Transferase-Inhibitor, Catechol-O-Methyltransferase-Inhibitor, Methyl-Donor, Methyl-Guanidine-Inhibitor
8.65	Benzeneethanamine, 2-fluoro-. beta.,3,4-trihydroxy-N-isopropyl-	229.1	$C_{11}H_{16}FNO_3$	1.53	17-beta-hydroxysteroid dehydrogenase-Inhibitor, Antiamyloid-Beta, AntiTGF-beta, Beta-2-Receptor-Agonist, Beta-Adrenergic Receptor Blocker, Beta-Andrenergic-Agents, Beta-Blocker, Beta-Galactosidase-Inhibitor, Beta-Glucuronidase-Inhibitor, ER-Beta-Binder, Anaphylactic, Antitumor, Arylamine-N-Acetyltransferase-Inhibitor, Decreases Norepinephrine Production, Down regulates of nuclear and cytosol androgen reuptake, GABA-nergic, Increases Natural Killer (NK) Cell Activity, Inhibits Production of Tumor Necrosis Factor
10.57	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-	496.3	$C_{27}H_{52}O_4Si_2$	0.52	NK
24.30	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	504.2	$C_{14}H_{44}O_6Si_7$	0.84	NK

Anti-inflammatory property

In vitro inhibition of inflammation pathway was studied by two methods namely by protein denaturation inhibition process and inhibition of RBC lysis. The extract inhibited the protein denaturation process with the IC_{50} value of 41.5 µg/mL (Figure 1c). The standard drug showed the inhibitory activity with the IC_{50} value of 5.16 µg/mL. Similarly, the RBC lysis was inhibited with increasing concentration of the extract. At the concentration of 1000 µg/mL, 82% inhibition of RBC lysis was observed (Figure 1d).

Anticancer activity of the extract

MTT assay

To study the anticancer activity, A549 lung cancer cell line was used. At 1000 µg/mL the 62.57% of the cell death was observed. In the phase contrast images, the cell morphology was changed and the cell density was reduced when compared to that of control (Figure 2a).

Ao/EtBr staining

In the control group, all the cells have a uniform morphology. In the extract treated group at the concentration of 500 µg/mL

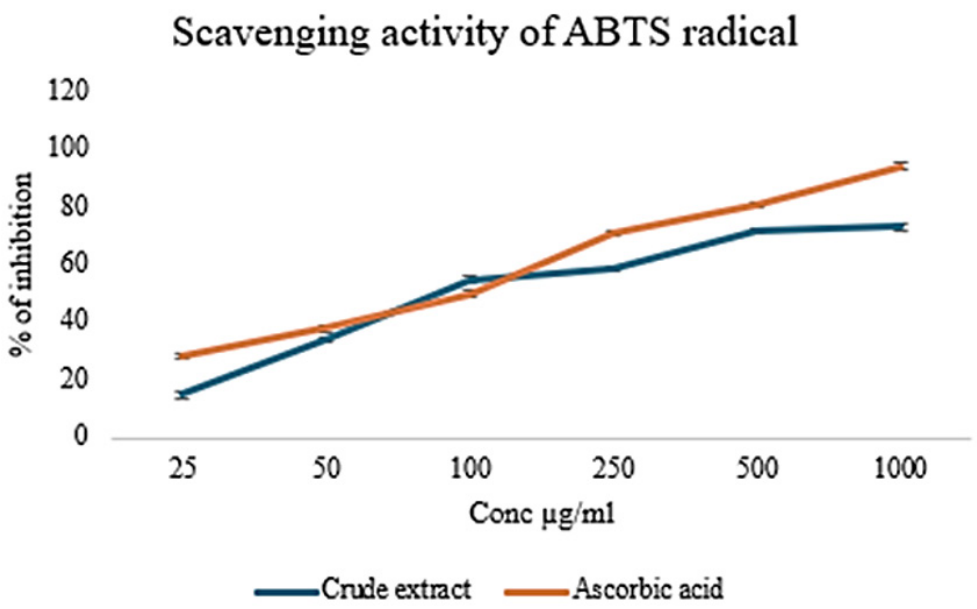

Figure 1a: ABTS* radical scavenging activity of the extract: Showing dose-dependent inhibition of oxidative stress with increase in concentration of the extract with ascorbic acid as standard.

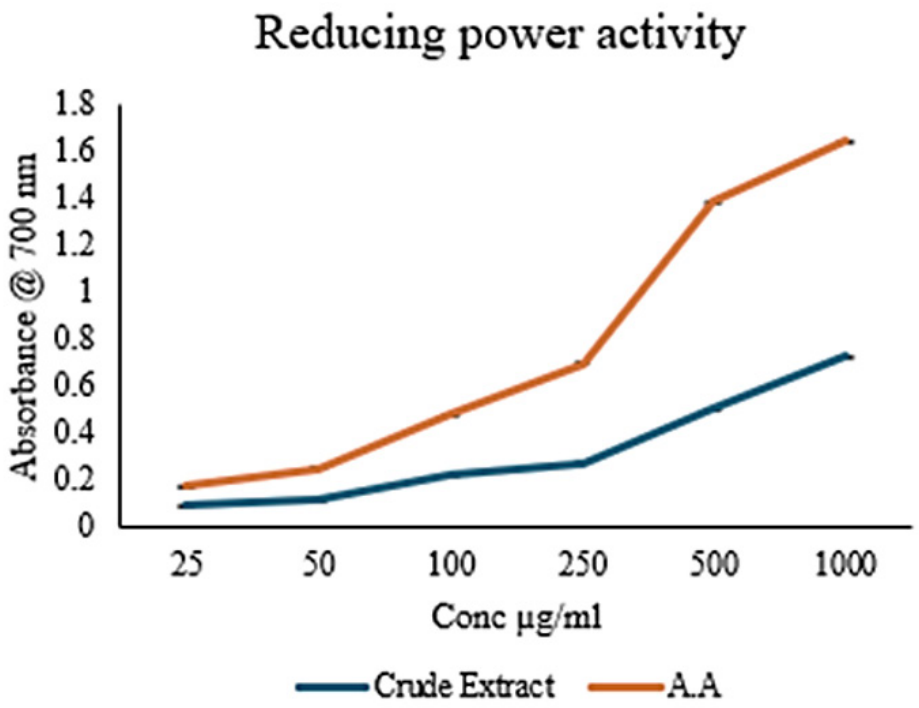

Figure 1b: Reducing power activity of the extract: Showing dose- dependent inhibition with increase in extract concentration and ascorbic acid as standard.

few cells lost its morphology and crescent shaped nucleus was observed. Cells treated with 1000 µg/mL showed more no of apoptotic cells was observed (Figure 2b).

DISCUSSION

Medicinal plants possess various bioactive compounds but the extraction protocol plays a vital role in the development of novel drugs. To analyse the types of compounds present in the samples, analytical tools such as GC-MS can be performed (Gomathi *et al.*, 2015). The results of the GC-MS analysis of the aerial parts of *Peristrophe bicalyculata* ethyl acetate extract, along with the possible medicinal role of each molecule of *Peristrophe bicalyculata* extract are tabulated in Table 1. Previous study showed the presence of seven bioactive compounds GC-MS analysis of ethanolic extract (Narayanan *et al.*, 2012). In the current study, 17 compounds with potent therapeutic value were

present in the ethyl acetate extract. The difference in the number of compounds between two study is mainly due to the solvent used in the extraction process (Hashmi *et al.*, 2013).

To study the therapeutic property of the ethyl acetate extract, antioxidant, anti-inflammatory and cytotoxic activity was studied. Synthesis of free radicals in the human system can pave the way to the development of various diseases. Scavenging of free radical by the medicinal plant in addition with different therapeutic property will helps to impede the progression of diseases (Diaz *et al.*, 2012). The current study assessed the free radical scavenging activity of the extract ABTS and reducing power assay. In a previous study, ethyl acetate extract showed the percentage of inhibition at the concentration of 1000 µg/mL was 55.45% to scavenge the DPPH radical (Arya and Metha., 2017). The result in the present study showed percentage of inhibition at the concentration of 1000 µg/mL was 73% to scavenge the ABTS radical. Iron is one of the important free radicals which reacts with macro molecules like lipid and generate lipid peroxidation (Gioti *et al.*, 2009).

Chelating the iron is very important antioxidant property and in the present study the chelating property of the crude extract was observed. Apart from antioxidant activity, significant *in vitro*

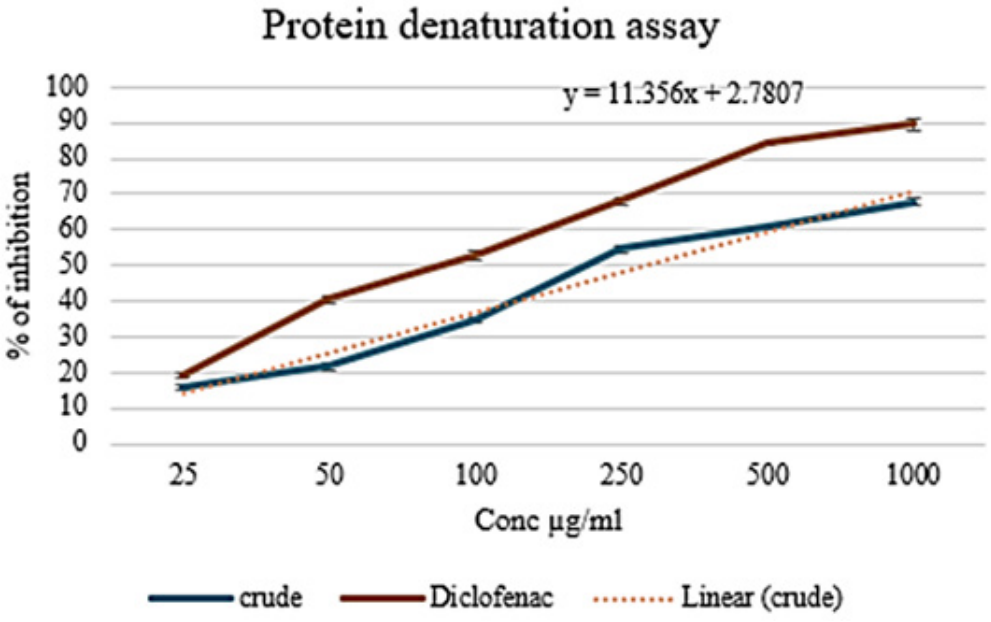

Figure 1c: Protein denaturation assay of the extract: Showing dose- dependent inhibition of inflammatory action with the IC_{50} value of 41.5 µg/mL comparing to the standard.

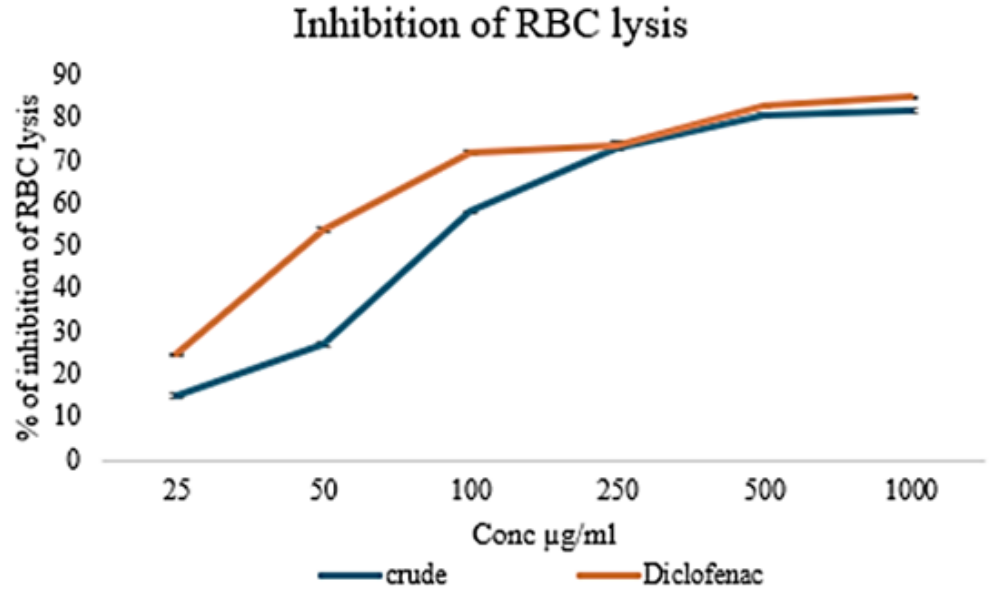

Figure 1d: Inhibition of RBC lysis by the extract: Showing dose- dependent inhibition of RBC lysis with increase in concentration with the IC_{50} value of 5.16 µg/mL.

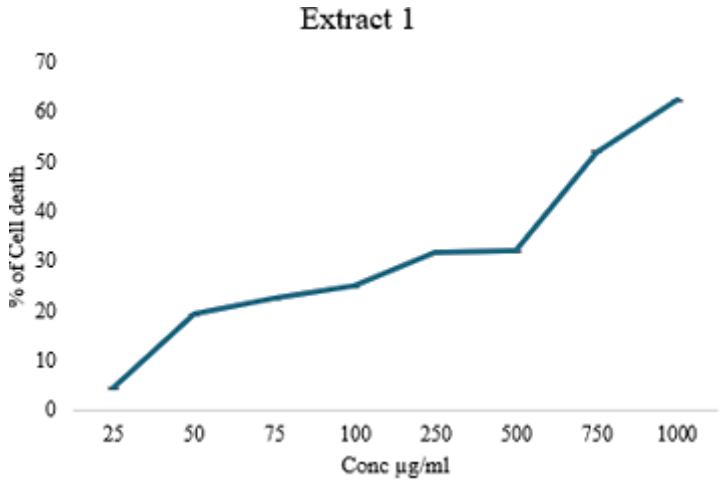

Figure 2a: MTT cell viability assay: Dose- dependent cell death was observed in crude extract treated A549 cell line with increase in concentration. The concentration of the extract is in X- axis and the % of cell death is in Y-axis.

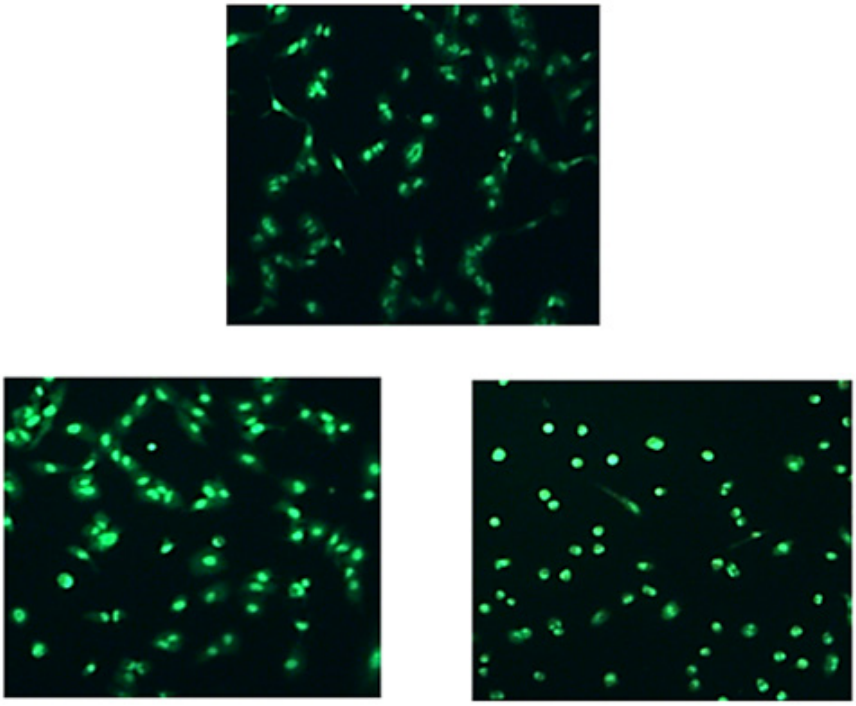

Figure 2b: Ao/EtBr staining: Reveals the presence of apoptotic and necrosis morphology in the cells at different concentration.

anti-inflammatory was observed in this study by two methods namely protein inhibition assay and RBC lysis inhibition assay. Human RBC cells and lysosomes membranes are similar in structure and function and during the inflammation process, lysosome membrane stabilization is very important to pervert the further progression of inflammation (Saleem *et al.*, 2011). In the present study, the stabilization of human RBC membrane was maintained by the crude extract of *Peristrophe bicalyculata*. Another mechanism of inflammation prevention is by inhibiting the protein denaturation. Denaturation of protein increases the production of auto antigens and increases the autoimmune disorders like rheumatic arthritis (Dharmadeva *et al.*, 2018). In the present study, crude extract inhibits the denaturation of protein caused by heat. Thus, the extract possesses anti-inflammatory activity by reducing the denaturation of protein and stabilizing the human RBC cells.

Apart from these biological activities, cytotoxic activity against cancer cells was also studied against lung cancer cell, A549. MTT assay is one of the consistent methods followed by many researchers to measure the cell viability (Ogbole *et al.*, 2017). Cytotoxic activity against the cancer cell line and the normal cell line is the preliminary screening to study the anticancer

properties (Nemati *et al.*, 2015). Ethyl acetate extract showed 62.57% cell viability measured by MTT assay.

Previous studies describe the anticancer activity of the leaf extract of this plant in cervical and foetal lung cancer cell line (Abdulazeez *et al.*, 2022). The current study was carried out in lung cancer cell line A549. To study the mechanism of cell death induced by the extract, A549 cells treated with Acridine orange and ethidium bromide stains. These two stains help to reveal the presence of apoptotic and necrotic cell population in the treated cells (Liu *et al.*, 2015). Staining of cell with Ao/EtBr also reveals that the extract induces apoptosis in the cell line. Further study in this extract will give the insights about the anti-cancer and anti-inflammatory activity of the plant.

CONCLUSION

Peristrophe bicalyculata is one of the important medicinal plants used in the traditional system of medicine. Though it has various therapeutic properties, scientific evidences were lacking. This study showed the ethyl acetate extract of aerial plant possess the various therapeutic properties including antioxidant, anti-inflammatory and cytotoxic activities. Further work has to be carried out to understand the molecular mechanism is warranted.

ACKNOWLEDGEMENT

The authors want to acknowledge the management for providing facility.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

GC-MS: Gas Chromatography- Mass Spectrometry; **IC_{50}:** Maximum Inhibitory Concentration; **MTT:** 3 -(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **Ao/EtBr:** Acridine Orange/Ethidium Bromide; **ABTS:** 2,2'-azino- bis(ethylbenzothiazoline-6-sulfonic acid); **DPPH:** 2,2-diphenyl- 1- picrylhydrazyl; **WHO:** World Health Organization; **HRBC:** Human Red Blood Cell; **PDB:** Protein Data Bank; **PBS:** Phosphate- Buffered Saline; **DMSO:** Dimethyl Sulfoxide; **EGFR:** Epidermal Growth Factor Receptos; **VEGF:** Vascular Endothelial Growth Factor; **PDL:** Programmed Death Ligand; **KRAS:** Kristen Rat Sarcoma Virus.

REFERENCES

Abdulazeez, M. A., Ibrahim, S., Amhe, D. A. *et al.* (2015) Bioassay guided fractionation and antihypertensive properties of fractions and crude extracts of P. bicalyculata. Acta Poloniac Pharmaceutica-Drug Research 7(2): 319–328.

Abdulazeez, M. A., Jasim, H. A., Bashir, M., Ross, K., & Fatokun, A. A. (2022). Peristrophe bicalyculata (Retz) Nees contains principles that are cytotoxic to cancer cells and induce caspase-mediated, intrinsic apoptotic death through oxidative stress, mitochondrial depolarisation and DNA damage. Biomedicine and Pharmacotherapy, 147, Article 112597. https://doi.org/10.1016/j.biopha.2021.112597

Abimbola, A. M., Baba, I. A., Yenusa, E. Z., Omanibe, S. J., & Oladimeji, I. H. (2013). Anti-trypanosomal effect of *Peristrophe bicalyculata* extract on *Trypanosoma brucei* brucei-infected rats. Asian Pacific Journal of Tropical Biomedicine, 3(7), 523–531. https://doi.org/10.1016/S2221-1691(13)60107-0

Al Hashmi, L. S., Hossain, M. A., Weli, A. M., Al-Riyami, Q., & AlSabahi, J. N. (2013). Gas chromatography-mass spectrometry analysis of different organic crude extracts from the local medicinal plant of *Thymus vulgaris* L. Asian Pacific Journal of Tropical Biomedicine, 3(1), 69–73. https://doi.org/10.1016/S2221-1691(13)60026-X

Arya, P. (2018). Antioxidant, phytochemical and antibacterial action of Himalayan medicinal herbs *Peristrophe bicalyculata* leaves extract against respiratory tract pathogens. International Journal of Pharmacy and Pharmaceutical Sciences, 10(3), 16–21. https://doi.org/10.22159/ijpps.2018v10i3.23961

Arya, P., & Mehta, J. P. (2017). Antioxidant potential of Himalayan medicinal plants *Angelica glauca, Alysicarpus vaginalis* and *Peristrophe bicalyculata*. International Journal of Current Microbiology and Applied Sciences, 6(7), 1892–1901. https://doi.org/10.20546/ijcmas.2017.607.226

Aye, M. M., Aung, H. T., Sein, M. M., & Armijos, C. (2019). A review on the phytochemistry, medicinal properties and pharmacological activities of 15 selected Myanmar medicinal plants. Molecules, 24(2), 293. https://doi.org/10.3390/molecules24020293

Dharmadeva, S., Galgamuwa, L. S., Prasadinie, C., & Kumarasinghe, N. (2018). *In vitro* anti-inflammatory activity of *Ficus racemosa* L. bark using albumin denaturation method. AYU (An International Quarterly Journal of Research in Ayurveda), 39(4), 239–242. https://doi.org/10.4103/ayu.AYU_27_18

Diaz, P., Jeong, S. C., Lee, S., Khoo, C., & Koyyalamudi, S. R. (2012). Antioxidant and anti-inflammatory activities of selected medicinal plants and fungi containing phenolic and flavonoid compounds. Chinese Medicine, 7, 1–9.

Djuichou Nguemnang, S. F., Tsafack, E. G., Mbiantcha, M., Gilbert, A., Atsamo, A. D., Yousseu Nana, W., Matah Marthe Mba, V., & Adjouzem, C. F. (2019). *In vitro* anti-inflammatory and *in vivo* antiarthritic activities of aqueous and ethanolic extracts of *Dissotis thollonii* Cogn. (Melastomataceae) in rats. Evidence-Based Complementary and Alternative Medicine: eCAM, 2019(1), Article 3612481.

Duke, J. A. (1994). Dr. Duke's phytochemical and ethnobotanical databases.

Farnsworth, N. R., Akerele, O., Bingel, A. S., Soejarto, D. D., & Guo, Z. (1985). Medicinal plants in therapy. Bulletin of the World Health Organization, 63(6), 965.

Gioti, E. M., Fiamegos, Y. C., Skalkos, D. C., & Stalikas, C. D. (2009). Antioxidant activity and bioactive components of the aerial parts of *Hypericum perforatum* L. from Epirus, Greece. Food Chemistry, 117(3), 398–404. https://doi.org/10.1016/j.foodchem.2009.04.016

Gomathi, D., Kalaiselvi, M., Ravikumar, G., Devaki, K., & Uma, C. (2015). GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of *Evolvulus alsinoides* (L.) L. Journal of Food Science and Technology, 52(2), 1212–1217. https://doi.org/10.1007/s13197-013-1105-9

Gomathi Priyadarshini, A. A. E., Anthony, J., Rao, M. R. K., Prabhu, K., Ramesh, A., & Krishna, V. (2017). The GC MS analysis of one medicinal plant, *Premna tomentosa*. Journal of Pharmaceutical Sciences and Research, 9, 1595–1597.

Janaki, C. S., Prabhu, K., Rao, M. R. K., Ramaiah, V., Dinkar, S., Vijayalakshmi, N., & Kalaivannan, J. (2021). The GC MS Analysis of ethyl acetate extract of *Merremia emerginata* Burm. F (*Ipomoea reniformis*). Indian J. Natl Sci., 12, 33638–33646.

Janakiraman, N., Johnson, M., & Sahaya, S. S. (2012). GC-MS analysis of bioactive constituents of *Peristrophe bicalyculata* (Retz.) Nees. (Acanthaceae). Asian Pacific Journal of Tropical Biomedicine, 2(1), S46–S49. https://doi.org/10.1016/S2221-1691(12)60128-2

Jayakumari, S., Prabhu, K., Rao, M. R. K., Kumaran, D., & Ramesh, A. (2017). The GC MS analysis of a rare medicinal plant *Aloe barbadensis*. Journal of Pharmaceutical Sciences and Research, 9(7), 1035.

Johnley, I. R., & Joshi, P. (2014). *In vitro* antioxidant activity of various extracts of the whole plant of *Peristrophe bicalyculata* forssk. Int. J. Pharm. Drug Anal, 2, 705–709.

Krishna, R. V., & Drisya, V. (2018). Preliminary phytochemical, antioxidant and antimicrobial studies of *Dicliptera paniculat*a, (forssk.) i. darbysh. Int. J. Dev. Res, 8, 23189–23192.

Kumar, M. H., Prabhu, K., Rao, M. R. K., Sundram, R. L., Shil, S., Kumar, M. S., & Vijayalakshmi, N. (2019a). The gas chromatography-mass spectrometry study of one medicinal plant, *Dodonaea viscosa* var. angustifolia. Drug Invention Today, 12(8), 1657–1661.

Kumar, M. H., Prabhu, K., Rao, M. R. K., Sundram, R. L., Shil, S., Kumar, M. S., & Vijayalakshmi, N. (2019b). The gas chromatography-mass spectrometry study of one medicinal plant, *Aristolochia indica*. Drug Invention Today, 12(12).

Liu, K., Liu, P. C., Liu, R., & Wu, X. (2015). Dual AO/EB staining to detect apoptosis in osteosarcoma cells compared with flow cytometry. Medical Science Monitor Basic Research, 21, 15–20. https://doi.org/10.12659/MSMBR.893327

Mgbeje, B. A. I., Essien, N. A., Iwara, I. A., Egbung, G. E., Igile, G. O., & Ebong, P. E. (2013). Lipid profile and hepatoprotective effects of combined leaf extracts of *Azadirachta indica* (Neem) and *Peristrophe bicalyculata* in Alloxan-induced diabetic rats. International Journal of Phytomedicine, 5(2), 159.

N, V., & Krishna Rao, M. R. (2019). The antioxidant studies of two medicinal plants, *Sphaeranthus indicus* and *Psophocarpus tetragonolobus*. Asian Journal of Pharmaceutical and Clinical Research, 12(1), 321–327. https://doi.org/10.22159/ajpcr.2019.v12i1.29951

Nemati, F., Dehpouri, A. A., Eslami, B., Mahdavi, V., & Mirzanejad, S. (2013). Cytotoxic properties of some medicinal plant extract from Mazandaran, Iran. Iranian Red Crescent Medical Journal, 15(11), Article e8871. https://doi.org/10.5812/ircmj.8871

Ogbole, O. O., Segun, P. A., & Adeniji, A. J. (2017). *In vitro* cytotoxic activity of medicinal plants from Nigeria ethnomedicine on rhabdomyosarcoma cancer cell line and HPLC analysis of active extracts. BMC Complementary and Alternative Medicine, 17, 1–10.

Ogunwande, I. A., Walker, T. M., Bansal, A., Setzer, W. N., & Essien, E. E. (2010). Essential oil constituents and biological activities of *Peristrophe bicalyculata* and *Borreria verticillata*. Natural Product Communications, 5(11). https://doi.org/10.1177/1934578X1000501125

Parameswari, P., Devika, R., & Vijayaraghavan, P. (2019). *In vitro* anti-inflammatory and antimicrobial potential of leaf extract from *Artemisia nilagirica* (Clarke) Pamp. Saudi Journal of Biological Sciences, 26(3), 460–463. https://doi.org/10.1016/j.sjbs.2018.09.005

Rao, M. R. K., & Lakshmi, N. V. (2018). Preliminary phytochemical and GC MS analysis of different extracts of *Sphaeranthus indicus* leaves. Indo American Journal of Pharmaceutical Sciences, 5(3), 1511–1520.

Rao, M. R. K., Vijayalakshmi, N., Prabhu, K., & Kumar, M. S. (2019). The gas chromatography-mass spectrometry study of *Moringa oleifera* seeds. Drug Invention Today, 12, 2172–2175.

Rashmi, G., Jaya, P., Hardik, P., Bhumi, M., & Shivani, A. (2010). *Peristrophe bicalyculata*-A review. Pharmacognosy Journal, 2(14), 39–45. https://doi.org/10.1016/S0975-3575(10)80070-7

Saleem, T. M., Azeem, A. K., Dilip, C., Sankar, C., Prasanth, N. V., & Duraisami, R. (2011). Anti-inflammatory activity of the leaf extacts of *Gendarussa vulgaris* Nees. Asian Pacific Journal of Tropical Biomedicine, 1(2), 147–149. https://doi.org/10.1016/S2221-1691(11)60014-2

Sofowora, A., Ogunbodede, E., & Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. African Journal of Traditional, Complementary and Alternative Medicines, 10(5), 210–229. https://doi.org/10.4314/ajtcam.v10i5.2

Srikanth, M., Devi, B., Kotirataiah, K., Ramanjaneyulu, M., Sulthana, P. N., & Suma, R. R. (2018). Phytochemical screening and. Journal of Agricultural and Food Chemistry, 50, 5294-5299.

Vijayalakshmi, N., & Rao, M. R. K. (2020). Preliminary Phytochemical and Antioxidant Studies of Leaf extracts of one Medicinal plant, Vitex negundo. Research Journal of Pharmacy and Technology, 13(5), 2167–2173. https://doi.org/10.5958/0974-360X.2020.00390.X

Vijayamuthuramalingam, U. D. K., Rajaram, R., Kuppusamy, K. M., Jonnalagadda, B., & Arokiasamy, S. (2017). Anti-hyperglycemic and antioxidant potential of *Croton bonplandianus*. Bail fractions in correlation with polyphenol content. Iranian Journal of Basic Medical Sciences, 20(12), 1390.

Yuvaraj, R., Rao, M. R. K., Prabhu, K., Lakshmisundram, R., Shil, S., Sathish Kumar, M., & Vijayalakshmi, N. (2019). The GC MS study of one medicinal plant, *Stachyterpheta indica*. Drug Invention Today, 12(9), 1665–1669.

Cite this article: Lakshmisundaram R, Kalaivani MK, Muruganandham L, Baskaran KPR, Muniyandi D, Rao MRK. Biological Profiling and Phytochemical Analysis of Peristrophe bicalyculata: Evaluating its Therapeutic Potential and Active Compounds. J Young Pharm. 2025;17(3):571-80.