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Research Article

Anti-Proliferative Effect of Methanolic Extract from Philodendron Burle-Marxii

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ABSTRACT

Cancer remains one of the leading cause of death and demands better therapeutic agents for its management. Different plant genus remains a source of chemotherapeutic agents. Philodendron is such a genus having immense medicinal potential. The species Philodendron bipinnatifidum is reported to have different pharmacological actions which is due to the presence of flavonoids and terpenoids. Philodendron burle-marxii is also a species in the Philodendron genus having similar phytochemicals. But biological potential of this species is not explored. In this study anti-proliferative effect of methanolic extract from Philodendron burle-marxii leaves were conducted through MTT assay, Chick chorioallantoic membrane assay, Comet assay. The methanolic extract has significant cytotoxic effect and it can also inhibit angiogenesis. The comet assay suggests the anti-proliferative actions may be due to its DNA damaging effect. This finding suggest the therapeutic of Philodendron burle-marxii.

INTRODUCTION

Cancer is a disease in which uncontrolled proliferation of cells and this proliferation can invade to other parts of the body, known as metastasis. More than hundred forms of cancers are existing. Signs and symptoms are varying with which part of body are affected. Even though advancement of chemotherapy and surgical interventions cancer remains one of the leading causes of death worldwide. So newer therapeutic agents are necessary for the better management of cancer.[1]

Herbal medicine plays major role in therapeutic system and many cancer medicines are derived from plant sources. The herbal medications which contains flavonoids and triterpenoids, have a significant medicinal potential.[2] Philodendron is such a promising plant genus which is native to the tropical regions of the America. It is a large genus comprising over 500 species. Philodendron species commonly known as Heart leaf, Heart leaf

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Ivy, Philodendron, Sweet heart plant. This genus of Plants are used as an ornamental plant and also act as an air purifier it grows both indoor and outdoor environment.[3] The species from the genus Philodendron is *Philodendron bipinnatifidum* which has been studied for its anti- inflammatory and antioxidant properties. The anti-inflammatory activity of *Philodendron bipinnatifidum* is attributed to its content of phenolic compounds and flavonoids. Traditionally, *Philodendron bipinnatifidum* has been utilized for its potential therapeutic properties in addressing conditions such as erysipelas, orchitis, and rheumatism. Philodendron plants contain a high concentration of terpenoids and flavonoids these constituents are responsible for the anti-proliferative effects. Jaqueline Scapinello *et.al.* in 2019 suggested another species of Philodendron such as *Philodendron burle-marxii* also have potential biological activities. The chemical constituent present in this species also suggest potential for anti-proliferative and anti-inflammatory activity.[4] But no scientific studies has been reported regarding the therapeutic potential of *Philodendron burle-marxii*. So, in this study we intend to evaluate the anti-proliferative action of the methanolic extract of the leaves of *Philodendron burle-marxii*.

MATERIALS AND METHODS

Collection, Identification, and Extraction of Plant Material

The leaves of *Philodendron burle-marxii* collected from Thiruvananthapuram and plant was authenticated from the Department of Botany, Kariavattom Campus, University of Kerala, Thiruvananthapuram, Kerala, India.

Preparation of Plant extract

The shade dried leaves were powdered and passed through the sieve number 10 and sieve number 66 then the powder retained in the sieve number 66 was separated and defatted with petroleum ether at a temperature of 50-60˚C using a Soxhlet apparatus. Thus, obtained marc after drying, extracted with methanol as solvent for up to 36 cycles. The methanolic extract of leaves of *Philodendron burle-marxii* (PBE) was concentrated at low temperature using Rotary evaporator.[5]

Preliminary Phytochemical Screening

The defatted PBE was subjected to qualitative test to identify various phytochemicals alkaloids, proteins, flavonoids, triterpenoids, tannins, steroids.[6]

Anti-proliferative Studies

MTT Assay[7]

The HCT- 116 cell line was obtained from the National Centre for Cell Sciences (NCCS) and was cultured using Dulbecco's modified Eagle's medium (DMEM). This cell line was maintained in a 25 cm^2 tissue culture flask containing DMEM enriched with 10% fetal bovine serum, L-glutamine, sodium bicarbonate and an antibiotic solution that included Penicillin (100 U/mL), Streptomycin (100 µg/mL) and Amphotericin B (2.5 µg/mL). Confluent monolayer of cells were suspended in 10% growth medium and seeded in a 96 well culture plate with 100µL cell suspension ($5x10^3$ cells/well) and incubated in a humidified 5% CO_2 incubator. Prepared the stock solution of PBE by dissolving 1mg of PBE in 0.1% DMSO. To ensure sterility, the sample solution was filtered via a 0.22 µm Millipore syringe filter. After 24 hours, the growth media was removed and PBE was introduced at concentrations of 100µg/mL, 50µg/mL, 25µg/mL, 12.5µg/mL, and 6.25µg/mL in DMEM. Each concentration was added in triplicate to relevant wells. Untreated cells were also kept as control. After incubating the samples for 24 hours, discarded the contents and added 30 µL of the MTT solution(5mg/ML) to each well designated for test and control groups. Incubated the plate at 37ºC in a humidified incubator with 5% CO_2 for 4 hours. Following the incubation period, removed the supernatant and introduced 100 µL of

DMSO to dissolve the formazan crystals. Absorbance readings were then taken using a microplate reader at a wavelength of 560 nm. The growth inhibition percentage was estimated using the following formula: Percentage viability = (Mean OD Samples /Mean OD of control group) x 100

Chick Chorioallantoic Membrane (CAM) Assay[8]

Three-day embryonated eggs were obtained from Regional Poultry Farm in Thiruvananthapuram. The eggs were gently wiped with 70% IPA. A light source was used to find the air sac and made a cut using forceps. The cut should be one centimeter in diameter. Using a syringe, carefully extracted the albumin. A plastic tape was used to cover eggs and kept in an incubator at 37˚C for 7 days. On the 10th day, the PBE was added at concentrations of 50, 100, 250, and 500µg/mL (**Fig.1**). The standard drug 5-flourouracil is added at the same concentration. A micropipette used to administer the drug to a sterilized filter paper disc that was kept on the CAM. Again, plastic tapes were used to cover eggs and placed in an incubator for three days. On the 13th day the shells were removed, and the contents poured in a petridish to compare the drug effect.

Fig.1. Photograph of embryonated egg on 10th day of CAM assay

Comet Assay[9]

HCT-116 cells were grown in a 6-well culture plate then incubated at 37ºC for 24 hours in a humidified 5% CO_2 incubator. Following confluence, cells were treated with PBE at LC_{50} concentration of 107.54 µg/mL and Doxorubicin and untreated cells served as the control. After 24hr of drug treatment cells were trypsinized, washed with fresh medium and utilized in the comet test. Microscope slides were covered with 1mL of 1% agarose and refrigerated at 4ºC. Cell suspensions (1×10^4/5-30µL) were mixed with 10µL of low melting point agarose, pipetted over the initial layer of agarose, covered with a cover slip, and kept at 4ºC. 1mL of 1% agarose was utilized as the final protective layer. After each stage, incubated the slides at 4ºC (for 20 minutes) to allow to set agarose. Slides were placed in a cold lysis solution containing 12.5 µM NaCl, 100 mM Na_2EDTA, 10 mM Tris base at pH 10, and 1% SDS. Just before use, 10% DMSO and 1% Triton X- 100 were added. After a 2-hour incubation, the slides were transferred to an electrophoresis buffer composed of 300 mM NaOH and Na_2EDTA at pH 13 for 20 minutes to allow the DNA to unwind. Electrophoresis was conducted in the same buffer with a voltage of 25 V (300 mA) for 20 minutes using an electrical supply. Finally, the slides were rinsed three times for 5 minutes each in a neutralization buffer (0.4 M Tris, pH 7.5), then dried and stained with 50 µL of ethidium bromide at a concentration of 20 µg/mL. Then slides were photographed using an Olympus CKX41 inverted epifluorescence microscope equipped with an Opitka Pro5 CCD camera. Comets were rated using Tritek's comet scoring software and statistically correlated.

RESULTS

Preliminary Phytochemical Screening

Investigation revealed the presence of alkaloids, phenolic compounds, tannins, triterpenoids and steroids.

MTT Assay

The cytotoxic potential of PBE was assessed by treating HCT-116 cells with various doses of PBE. The percentage viability of HCT-116 cells after

24-hour treatment with standard drug Doxorubicin and PBE were summarized in the **Fig.2**. The PBE inhibited the proliferation of cancer cells. The LC_{50} of PBE was found to be 107.54µg/mL.

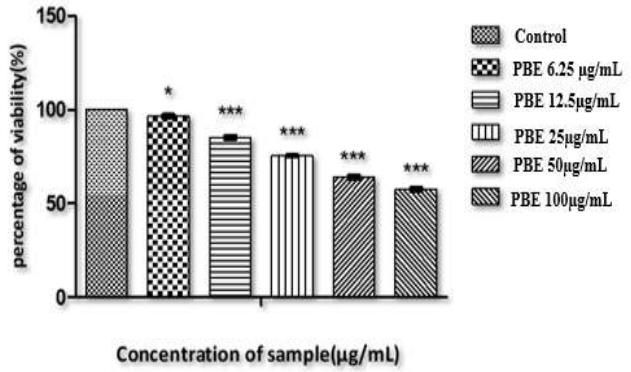

Fig.2. Graphical illustration of the anticancer effect of PBE on the HCT-116 cell line using the MTT assay.

The results were represented as Mean+/- SE. One-way ANOVA and Dunnets test were performed to analyse data. ***$p < 0.001$ compared to control groups, *$p < 0.1$ compared to control groups. PBE-Methanolic extract of leaves of *Philodendron burle-marxii*.

Chick Chorioallantoic membrane (CAM) assay:

The angiogenesis inhibitory activity PBE was examined by CAM assay. 5- fluorouracil was used as the positive control. Macroscopic examination of control showed normal angiogenesis with dendritic branching pattern of blood vessel formation. They induced abundant blood vessel sprouting. However, the treatment with 50, 100, 250, 500µg/mL concentrations of PBE produced a significantly visible inhibition in angiogenesis and branching pattern of blood vessel formation was very poor in the given concentration. The 5-fluorouracil with each concentration showed a clear-cut inhibition of angiogenesis (**Fig.3**). The anti-angiogenic effect produced clearly visible in the photograph shown. These results indicates that methanolic leaf extract of *Philodendron burle-marxii* may have anti- angiogenic activity.

Comet Assay

The microscopic images of PBE, Doxorubicin (DXR) and control photographed using inverted epifluroscent microscope is shown in **Fig.4**. **Fig.5** shows the comet length of PBE and the standard DXR. The PBE produced a significant DNA damage in comparison to control which is less than that of DXR.

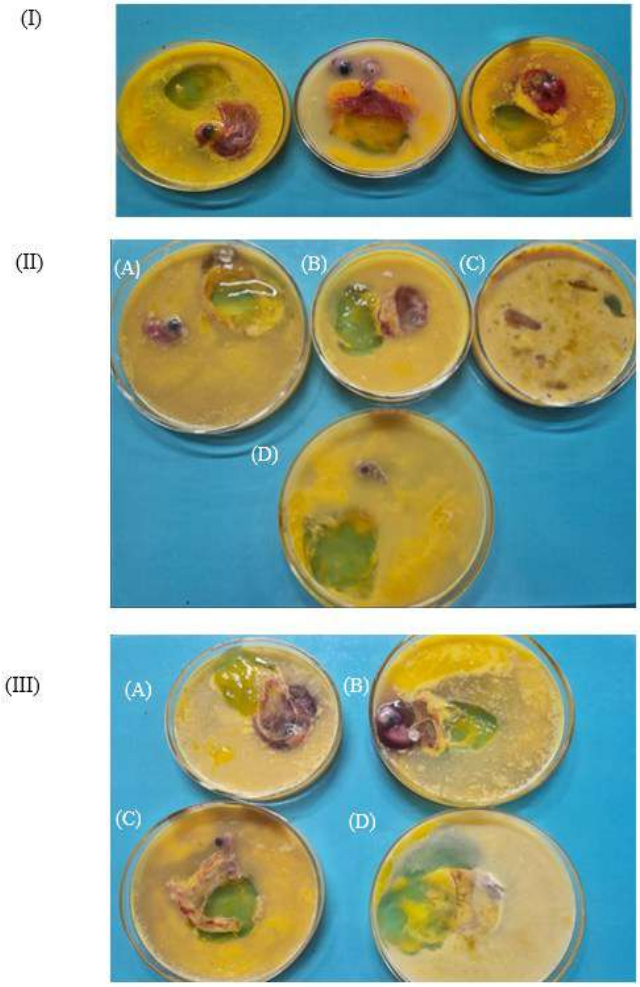

Fig.3. Photograph of embryonated egg after 13 days of CAM Assay (I) Control (II) Positive control 5- fluorouracil with concentration A- 50µg/ml, B-100µg/ml, C- 250µg/ml, D-500µg/ml (III) PBE in the concentrations of A-50µg/ml, B-100µg/ml, C-250µg/ml, D-500µg/ml

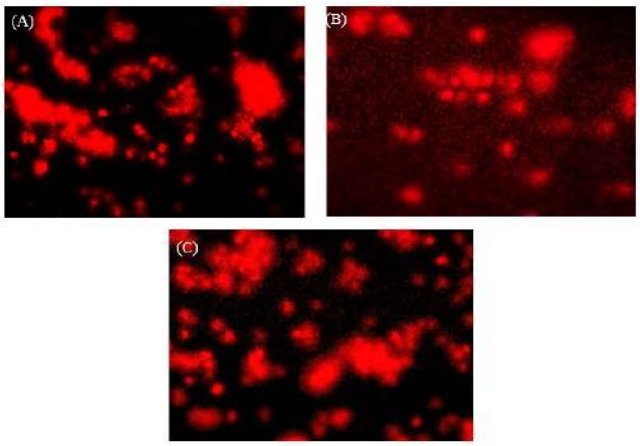

Fig.4. Microscopic images of PBE, DXR and control photographed using inverted epifluroscent microscope (A) control (B) treated with doxorubicin (9.93µg/mL) (C) treated with PBE (107.54µg/mL)

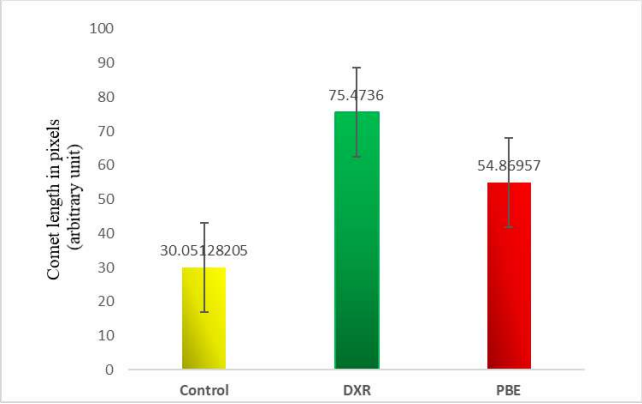

Fig.5. Comparison of comet length between control, DXR and PBE DXR- Doxorubicin PBE- Methanolic extract of *Philodendron burle-marxii* extract

DISCUSSION

The current study evaluated the anti-proliferative effect of methanolic extract from leaves of *Philodendron burle-marxii*. Phytochemical analysis of *Philodendron burle-marxii* demonstrated the presence of alkaloids, phenolic compounds, tannins, triterpenoids and steroids. In this study the PBE showed significant inhibition of percentage of viability. The IC_{50} value of PBE was found to be 107.78±5.46µg/mL. This IC_{50} value indicates a significant cytotoxicity. But the IC_{50} value is not comparable to DXR. This may be due to the crude nature of extract more purification can resolve this.[10] Chick chorioallantoic membrane assay was done in the PBE for anti-angiogenic potential. The PBE showed significant anti-angiogenic activity. Here 5- fluorouracil was used as positive control. Compare to the positive control the PBE has significant anti-angiogenic effect as PBE inhibit the cell proliferation and thus angiogenesis. The DNA damage was studied by Comet assay in HCT-116 cell line. Comet length were identified to assess DNA damage. A longer the comet length it indicates more DNA damage. PBE produced a significant DNA damage in comparison to control. This study gives an insight into the possible mechanism of action. The anti-proliferative effect of the PBE may be due to this DNA damaging effect.

CONCLUSION

The methanolic plant extract *Philodendron burle-marxii*. has anti-proliferative potential. The results from assays such as MTT, Comet, Chick chorioallantoic membrane assay highlight the extract's potential to produce anti-proliferative action and this action may be due to producing DNA damage. These findings suggest that the extract may serve as valuable source for developing therapeutic agents targeting cancer.

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Conflict of Interest

All the authors have no conflict of interest.

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