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# Preliminary Screening of Hydro-ethanolic Extract of *Pothos scandens* L for its Potential Anticancer Activity

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***Abstract :*** *Pothos scandens L is a medicinal aroid which has been used traditionally by people for curing many diseases including epilepsy, convulsions and cancer. The present study was carried out to screen the potential anticancer activity of 50% hydro-ethanolic extract of Pothos scandens L (HEEPS). In the present study evaluation of phytochemical constituents of aerial parts of Pothos scandens L using various extracting solvents viz- petroleum ether, chloroform, acetone, methanol, ethanol and water, its antioxidant activity using Ferric Reducing Antioxidant Power (FRAP) assay and evaluation of its potential cytotoxic activity using LDH leakage analysis and DNA fragmentation assay in MCF-7 breast cancer cell lines were carried out. The results revealed that presence of phyto-constituents were more in ethanolic extract when compared to others. The FRAP value of HEEPS was found to be 187.49 ± 11.32 mmol Fe2+/g extract. The LDH leakage assay showed significant cytotoxic activity against MCF-7 cell lines. Further visible fragmentation of DNA was observed in MCF-7 cell lines treated with HEEPS. From the results obtained, it can be concluded that Pothos scandens can be considered as a potential candidate for anticancer drug research.*

***Keywords:*** *Anticancer, FRAP assay, LDH leakage assay, MCF-7 breast cancer cell lines, Pothos scandens L.*

## 1. Introduction

In the current world the use of medicinal plants and traditional health systems is gaining importance in solving health related problems. From the time immemorial plants as been the source of medicinal preparations in the form of simple raw material or in the refined form of crude extracts, etc [1]. In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products especially those derived from plants [2]. Plant derived drugs are used to cure mental illness, skin diseases, tuberculosis, diabetes, jaundice, hypertension and cancer. Plant derived drugs came into use in the modern medicine

through the uses of plant material as indigenous cure in folklore or traditional systems of medicine [3]. Cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives every year [4]. It is a serious clinical problem that possesses significant social and economic challenges to the health care system [5]. Though there are many therapeutic strategies including chemotherapy to treat cancer, high systemic toxicity and drug resistance limit the successful outcomes in most cases. Moreover cancer treatment is usually accompanied by diverse side effects to different body organs [6]. Hence there is a worldwide trend to go back to natural resources (medicinal plants) which are therapeutically effective, culturally acceptable and economically affordable [7]. The present study was carried out to screen the phytochemical compounds present in various extracts of *Pothos scandens* L, itsvantioxidant activity and its potential cytotoxic activity.

## 2. Materials and methods

### *2.1 Plant collection and authentication*

Pothos scandens were collected from in and around Palai, Kottayam, Kerala. They were identified and certified by the Taxonomist, Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India (Plant identification no.-BSI/SRC/5/23/2013-14/Tech/685).

### *2.2 Plant extraction and phytochemical analysis*

The aerial part of Pothos scandens were shade dried and ground to coarse powder. Air dried and pulverized plant material was weighed out and packed into a thimble, which was in turn placed in a soxhlet extractor and was extracted using different solvents viz., petroleum ether, chloroform, acetone, methanol, ethanol and water using soxhlet apparatus. The extracts were condensed to dryness using rotary evaporator and then used for the qualitative analysis of phytochemicals.

### *2.3 Qualitative phytochemical analysis*

The qualitative analysis to detect the presence of phytochemicals present in various extracts of *Pothos scandens* were preformed according to Mukherjee, 2002 [8]

### *2.4 Preparation of 50% hydro-ethanolic extract of Pothos scandens L(HEEPS)*

The aerial part of *Pothos scandens* were shade dried and ground to coarse powder. Air dried and pulverized plant material was weighed out and packed into a thimble, which was in turn placed in a soxhlet extractor and extracted using 50% ethanol for 48 hours. The extract obtained was filtered and condensed to dryness using rotary evaporator and stored at $-20^0$C for further studies.

### *2.5 Ferric reducing antioxidant power (FRAP) assay*

Ferric reducing antioxidant power assay was performed according to the method of Benzie and Strain, 1996 [9]. The FRAP reagent contained 2.5 ml of a 10 mM TPTZ solution in 40 mM HCl, 2.5 ml of 20 mM $FeCl_3.6H_2O$ and 25 ml of 300 mM acetate buffer (pH 3.6). It was freshly prepared and warmed at 37°C. 900µl FRAP reagent was mixed with 90µl water

and 10µl of the extract. The reaction mixture was incubated at 37°C for 30 minutes and the absorbance was measured at 593 nm.

*2.6 Maintenance of cell culture*

MCF-7 breast cancer cell lines and L929 (Fibroblast cells) cell lines were obtained from National Center for Cell Sciences (NCCS), Pune and was maintained in Dulbecco's modified eagles media (HIMEDIA) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37°C in 5 % $CO_2$ in a humidified atmosphere in a $CO_2$ incubator (NBS, EPPENDORF, Germany) and subcultured once confluency is reached.

*2.7 Evaluation of cytotoxic activity of Pothos scandens L*

*2.7.1 LDH leakage assay*

MCF-7 cells cultured in Dulbecco's modified Eagles media were grown to 60-70% confluency and treated with extracts of *Pothos scandens* at a final concentration of 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml and100 µg/ml from a stock of 1mg/ml and incubated for 24 hours. LDH leakage assay was performed as per the methods described by Andrey and Erich [10]. Briefly, 2.7 ml potassium phosphate buffer, 0.1ml NADH solution and 0.1ml sodium pyruvate solution were pipetted into a cuvette (light path : 1cm). Equilibrated at 25oC for about 5 minutes. 0.1 ml of sample was added and mixed. Recorded the decrease of absorbance at 340nm in a spectrophotometer thermostated at 25oC and calculated the ΔA/ minute using the linear portion of the curve (ΔA5min). The blank solution was prepared by adding enzyme dilution buffer instead of sample (ΔAomin).
Calculation:

Activity can be calculated by,

$$\text{Volume activity (U/ml)} = \frac{(\Delta A_{5\text{min}} - \Delta A_{0\ \text{min}}) \times 3 \times \text{df}}{6.2 \times 0.1}$$

*2.7.2 DNA fragmentation analysis*

MCF-7 cells cultured in Dulbecco's modified Eagles media were grown to 60-70% confluency and treated with extracts of *Pothos scandens* at a final concentration of 90.18µg/ml ($IC_{50}$) from a stock of 1mg/ml and incubated for 24 hours.

Genomic DNA was isolated using Purelink Genomic DNA isolation kit (Genomic DNA mini kit, Cat 1820- Invitrogen, USA). The cells after treatment were trypsinized and were then centrifuged at 6000 rpm and the pellet was collected. To it 180 µl PureLink™ Genomic Digestion Buffer and 20 µl Proteinase K was added and incubated at room temperature for 30 minutes.then added 20 µl RNase A to lysate, mixed well by brief vortexing, and incubated at room temperature for 2 minutes ,to it 200 µl PureLink™ Genomic Lysis/Binding Buffer was added and mixed well by vortexing to yield a homogenous solution followed by the addition of 200 µl 96-100% ethanol to the lysate, mixed well by vortexing to yield a homogenous solution. The whole lysate (~640 µl) prepared with PureLink™ Genomic Lysis/Binding Buffer and ethanol was added to the spin column, and centrifuged at 12,000 rpm for 1 minute at room temperature.The collection tube was

discarded and the spin column was placed into a clean PureLink™ Collection Tube supplied with the kit. To it Added 500 µl of Wash Buffer 1 prepared with ethanol to the column. The column was centrifuged at 12,000 rpm for 1 minute at room temperature. Discarded the collection tube and placed the spin column into a clean PureLink™ collection tube supplied with the kit. Then added 500 µl Wash Buffer 2 prepared with ethanol to the column. Centrifuged the column at maximum speed for 3 minutes at room temperature. Discarded the collection tube and placed the spin column in a sterile 1.5-ml microcentrifuge tube, to it added 50 µl of PureLink™ Genomic Elution Buffer and incubated at room temperature for 1 minute and centrifuged the column at maximum speed for 1 minute at room temperature to recover DNA.

*2.7.2.1 Agarose gel electrophoresis*

Agarose gel electrophoresis is a method for separating and visualizing DNA fragments. The fragments are separated by charge and size and move through agarose gel matrix, when subjected to an electric field. The electric field is generated by applying potential across an electrolyte solution (buffer).When boiled in an aqueous buffer, agar dissolve and upon cooling solidifies to a gel. 1.5% agarose gel was prepared in 1x TE buffer and melted in hot water bath at 90◦C.Then the melted agarose was cooled down to 45◦C. 6µl of 10 mg/ml of ethidium bromide was added and poured in to gel casting apparatus with the gel comb. After setting, the comb was removed from the gel. The electrophoresis buffer was poured in the gel tank and the platform with the gel was placed in it so as to immerse the gel. The gel was loaded with the samples and run at 50 V for 30 minutes. The stained gel was visualized using a gel documentation system (E gel imager, Invitrogen).

*2.8 Statistical analysis*

All experiments were performed in triplicate (n=3) and the results were expressed as mean ± standard deviation. Statistical analysis was carried out using SPSS 16.0.

## 3. Results and discussion

*3.1 Qualitative phytochemical analysis*

Secondary metabolites obtained from plants have proved to be an excellent reservoir of a wide variety of compounds with medicinal properties. The result of preliminary phytochemical analysis of *Pothos scandens* L using various extractive solvents are given in Table 1. From the analysis, it was found that ethanolic extract contained more amount of phytochemicals than the other solvents tested. The result indicated that the phytochemicals were present in the following order - ethanol>acetone>aqueous>methanol>petroleum ether>chloroform.

Table 1: Preliminary phytochemical analysis of *Pothos scandens* L

| TESTS | EXTRACTIVE SOLVENTS | | | | | |
|---|---|---|---|---|---|---|
| | PET | CHL | ACT | **ETH** | MET | AQS |
| Alkaloids | + | - | + | **+** | - | - |
| Flavonoids | - | + | + | **+** | + | + |
| Saponins | + | - | - | **+** | - | + |
| Carbohydrates | + | + | + | **+** | + | + |
| Protein | - | - | - | **+** | + | + |
| Phenols | + | - | + | **+** | + | + |
| Sterols | - | + | + | **+** | - | + |
| Glycosides | + | + | + | **+** | + | - |
| Tannins | + | - | + | **+** | + | + |
| Thiols | - | - | + | **+** | - | - |
| %Yield | 1.54% | 1.91% | 4.5% | **8.27%** | 8.36% | 16.41% |

Symbol (+) and (-) indicates the presence and absence respectively of plant constituents with respect to various extractive solvents in the increasing order of polarity. Experiments were carried out in multiples of three sets for each test. Further, it was also evident from the result (Table 1) that aqueous extract gave more percentage yield when compared with other solvents followed by ethanolic extract. Petroleum ether extract gave the least percentage of yield.

*3.2 Ferric reducing antioxidant power*

FRAP assay is a simple, inexpensive, speedy and highly reproducible. The ability of plant extract to reduce ferric ion was determined using FRAP assay. FRAP value of HEEPS was found to be 187.49 ± 11.32 mmol $Fe^{2+}$/g extract.

*3.3 LDH leakage assay*

Lactate dehydrogenase is used as a quantitative marker enzyme for intact cell. Measurement of LDH leakage is an important test for severe cellular damage. LDH is a a cytoplasmic enzyme released when cell membrane damages, is measured in cell culture supernatants [11]. Figure 1 shows the cytotoxic activity of *Pothos scandens* assayed using LDH leakage assay. From the result it is evident that HEEPS was having significant cytotoxic activities which lead to severe damage to the membrane integrity of MCF-7 cell lines and further the results also showed that the cytotoxic activity was in a dose dependent manner.

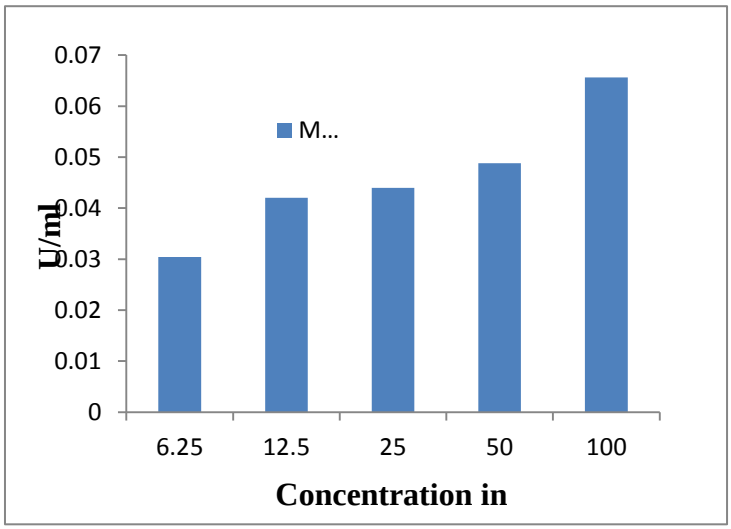


Figure 1: LDH Leakage assay of MCF-7 cells treated with 6.25, 12.5, 25, 50 and 100µg/ ml of 50% hydro-ethanolic extract of *Pothos scandens*.

*3.4 DNA fragmentation analysis*

Degradation of DNA by endogenous DNAses is onof the biochemical hall marks of of apoptosis. DNA fragmentation forms a ladder pattern that can be used to distinguish between apoptosis and necrosis [12, 13]. The result of DNA fragmentation analysis carried out using 50% hydro-ethanolic extract of *Pothos scandens* on MCF-7 cell lines is shown in Figure 2. From the results obtained it was found that in MCF-7 cells treated with vehicle for 24 hour, DNA fragmentation was not detectable (Figure 2, Lane 3). However, DNA fragmentation was apparent in MCF-7 cells treated with *Pothos scandens* extract for 24 hours (Figure 2, Lane 2) which indicated that the cytotoxic effect of the plant extract was through apoptosis.

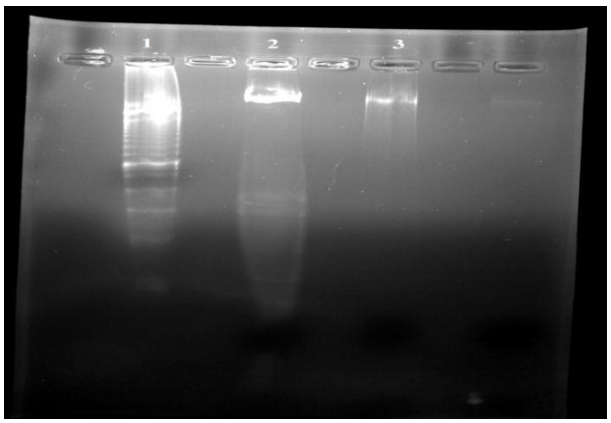


Figure 2: HEEPS induced DNA fragmentation in MCF-7 cell lines.

Lane 1: 100-bp DNA marker; Lane 2: MCF-7 cell lines treated with 90.18µg/ml ($IC_{50}$) of HEEPS for 24 hours and Lane 3: Untreated MCF-7 cells cultured in the presence of vehicle for 24 hours. Isolated DNA was loaded into agarose gel and electrophoresed.

## 4. Conclusion

From the present study it can be conclude that, the *Pothos scandens* possesses significant antioxidant and cytotoxic as well as apoptotic activity. Basing on these promising results, further studies may be carried out to identify the potency of this plant against cancer and the active components responsible for these activities.

## REFERENCES

1. Farnsworth NR. and Soejarto DD. Global importance of medicinal plants. In: Akerele, O., Heywood, V., and Synge, H. (Eds.), *The Conservation of Medicinal Plants.* Cambridge University Press, Cambridge: 1991, 25-51.
2. SchwartsmannnG, Ratain MJ, Cragg GM, Wong JE and Saijo N *et al*. Anticancer drug discovery and development throughout the world. *J. Clin. Oncol, 20*, 2002, 47S-59S.
3. Perumal SR. and Ignacimuthu S. Screening of 34 Indian medicinal plants for antibacterial properties. *J. Ethnopharmacol*., *62*, 1998, 173-182.
4. Karthikeyan R, Karthigayan S, Sri Balasubashi M, Vijayalakshni S and Balasubramanian T. Antitumor effect of snake venom (Hydrophis spiralis) on ehrlich ascites carcinoma bearing mice. *Int. J. Cancer Res,3*, 2007, 167-173
5. Guo J and Wang MH. Extract of Ulmus davidiana planch barks induced apoptosis in human hepatoma cell line HEPG2. *Experimental and clinical science journal. 8*, 2009, 130-137
6. Saalu LC, Ajayi GO, Adeneye AA, Imosemi IO and Osinubi AA. Ethanol seed extract of grapefruit (Citrus paradisi) as an effective attenuator of doxorubicin-induced oxidative stress in the rat heart. *Int. J. Cancer Res, 5*, 2009, 44-52.
7. Jeyachandran R A, Mahesh and Cindrella L. DEN- induced cancer and its alleviation by *Anisomeles malabarica* (L) R. Br. ethanolic leaf extract in male albino mice. *Int. J. Cancer. Res., 3*, 2007, 174-179
8. Mukherjee PK . Quality control herbal drugs. 1st edition, Business Horizons, New Delhi, 2002, pp-255-378.
9. Benzie IFF and Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant power" The FRAP assay. *Anal Biochem*. 1996; 239: 70-76.
10. Andry V Kuznetsov and Erich Gnaiger, Laboratory protoco.l Lactate dehydrogenase cytosolic marker enzyme, Mitochondrial physiology network, 2006;8.18:1-7
11. Saad B, Dakwar S, Said O, et al. Evaluation of medicinal plant hepatotoxicity in co-cultures of hepatocytes and monocytes. *Evidence-Based Complementary and Alternative Medicine, 3(1)*, 2006,:93–98.
12. Saraste A, Pulkki K. Morphologic and biochemical hallmarks of apoptosis. *Cardiovasc. Res*. *45*, 2000, 528–537.
13. Elmore S. Apoptosis: A review of programmed cell death. *Toxicol. Pathol. 35*, 2007, 495–516.