Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(8):108-112
(http://derpharmachemica.com/archive.html)

Preliminary phytochemical screening and comparison of anticancer potential of ethanolic and aqueous extracts of *Pajanelia Longifolia* (Willd) K. Schum

Gini. E. J[1], Sivakumar T[2] and Kuppuswamy S[3]

[1]*Chemists College Of Pharmaceutical Sciences And Research, Varikoli, Kerala, India*
[2]*Department Of Pharmacy, Annamalai University, Annamalai Nagar, Chidambaram,Tamilnadu, India*
[3]*Department Of Pharmaceutics ,Nirmala College Of Pharmacy, Muvattupuzha, Kerala, India*

ABSTRACT

The present study includes the preliminary phytochemical screening and evaluation of the anticancer potential of ethanolic as well as aqueous extract of whole plant Pajanelia longifolia (Willd) K.Schum. Anticancer activity of the whole plant were investigated by standard MTT assay method using Hacat and MCF-7 cell lines and the IC50 values were calculated. The invitro cytotoxic effect of ethanolic and aqueous extracts of the whole plant Pajanelia longifolia (Willd) K.Schum were identified. Among this ethanolic extract was found to be more effective against Hacat and aqueous extract possess more activity against MCF- 7. The present study concludes that both the extracts of whole plant of Pajanelia longifolia (Willd) K.Schum acquired anticancer activity against Hacat and MCF-7 cell lines.

Key Words : *Pajanelia longifolia* (Willd) K.Schum ,Soxhlet extraction, cytotoxic, MTT assay, linear regression analysis.

INTRODUCTION

Cancer continues to be the third leading cause of the death worldwide preceded by cardiovascular and infectious disease. Cancer is characterized by rapid and uncontrolled proliferation of abnormal cells spread throughout the body and eventually cause death of the host. Due to high systemic toxicity and drug resistance of chemotherapeutic agents, many of the cancer patients are in search of an alternative method for the treatment . Plants have been used long years back for the treatment of cancer and more than 60% of currently used anti-cancer drugs are obtained from various natural sources[1;2;3].

Pajanelia longifolia (Willd) K.Schum belong to the family Bignoniaceae, tall deciduous tree, scaly bark, linearly lenticellate, blaze whitish brown. Leaves are compound, imparipinnate, to 120cm, glabrous, leaflets opposite, 9-14 pairs with terminal odd one, lamina 8-24x3-10cm, ovate, apex acuminate, base asymmetric and margin entire. Inflorescence panicle; flowers purple outside and yellow within, petals wooly along margin. Fruits are capsule, 30-50x6-8cm, brown, 2 winged; seeds many, flat, with membraneous wing[4].

The MTT assay method was first introduced by Mosmann [5] and then developed by others [6]. It is very sensitive, quantitative and reliable colorimetric method that measures viability, proliferation and activation of cells. This method is mainly based on the capacity of the cellular mitochondrial dehydrogenase enzyme in living cells to reduce the yellow water-soluble substrate 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide(MTT) in to a dark blue/purple formazan product which is insoluble in water. The amount of formazan produced is directly proportional to the cell number in a range of cell lines. The results are consistent with those obtained from 3H-thymidine uptake assays. The MTT assay has greater applicability in the detection of cells which are not dividing but are still metabolically active. Therefore it can be used to distinguish proliferation and cell activation. Moreover, MTT

reduction occurs only in metabolically active cells, marked changes in metabolic activity can result in significant changes in results, despite the fact that the number of viable cells remains constant. IC50 value is used to determine the concentration of an anti-cancer drug that kills half of the cells in a cancer cell line and the value calculated by non-linear regression analysis[7].

MATERIALS AND METHODS

The plant *Pajanelia longifolia* (Willd) K.Schum was collected from the western guards of Kerala, authenticated and identified by Dr. Abraham Mathew,Professor, Dept.of Botany, St.Peters college, Kolenchery,Kerala and the extraction was carried out using Soxhlet apparatus.Extraction involves the separation of medicinally active portions of animal or plant tissues from the inactive components by the use of selective solvents.In Soxhlet apparatus a small volume of menstruum is made to circulate through the extractor containing the drug, again and again by the method of evaporation and subsequent condensation of menstrum.[8].

The invitro cytotoxic activity were performed using MTT assay method. MTT is a colorimetric assay that measures proliferation, viability and activation of cells[5]. MCF-7 (breast cancer cell line) and Hacat cell lines (keratinocytes) was obtained from NCCS, Pune, India and was maintained in Dulbecco's modified eagles medium (Gibco, Invitrogen).

The cell lines were 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 37^0C in a humidified 5% CO_2 incubator.

Extraction and Phytochemical Analysis

The whole parts of the plant *Pajanelia longifolia* (willd.) K.Schum was gabled for removal of adulterants and then pulverized. It was air dried at room temperature and 150gm of the pulverized part was exhaustively and sequentially extracted using Petroleum ether, Chloroform, Ethyl acetate, Ethanol and distilled water respectively with the Soxhlet extractor (gradient extraction). The extracts were concentrated in vacuum, weighed and properly labelled and stored in refrigerator until use [2].

Various qualitative tests were performed on the whole part of the plant extracts of *Pajanelia longifolia* (Willd.) K. Schum for the identification of different phytoconstituents [9; 10].

***In vitro* cytotoxic activity of *Pajanelia longifolia* (Willd) K. Schum whole plant**

The cells were trypsinized for 2 minutes and passed to T flasks in complete aseptic conditions. Extract was added to grown cells at the concentrations of 6.25 - 100µg/ml from a stock of 10mg/ml in 0.1% DMSO and incubated for 24 hours. Dilution of stock solutions was prepared in culture medium yielding final extract concentrations with a final DMSO concentration of 0.1%. This concentration of DMSO did not affect the cell viability. Control cells were incubated in culture medium only. Every concentrations of the plant extract were in triplicates on the same cell batch.

The % difference in viability was determined by standard MTT assay after 24 hours of incubation. The cell culture suspension was washed with 1x PBS and then 30 µl of MTT solution was added to the culture. Then incubated at 370°C for 3 hours. MTT was removed by washing with 1x PBS and 200µl of DMSO was added to the culture. Incubation was done at room temperature for 30 minutes until the cell got lyses and colour was obtained. The solution was transferred to centrifuge tubes and centrifuged at 4000rpm for 2minutes to precipitate cell debris. Optical density was examined at 540nm using DMSO as blank in an ELISA reader.

% viability = (OD of Test/ OD of Control) X 100.

Experimental results were expressed as mean ± SD. All measurements were replicated three times. The IC50 values were calculated from linear regression analysis.

RESULTS AND DISCUSSION

The plant used in study was identified as *Pajanelia longifolia* (willd) K.Schum and quantitative analysis showed the presence of saponins, flavonoids, carbohydrates, tannins and the results were in Table 1. Maximum phytoconstituents were found to be present in aqueous and ethanolic extracts of *Pajanelia longifolia* (willd) K.Schum and hence they were selected to perform the invitro cytotoxic studies.

Table 1 Phytochemical screening of *Pajanelia longifolia* (Willd.) K.Schum extracts

SL NO	Test	*Pajanelia longifolia* (Willd.) K.Schum				
		PET.Ether	Ethyl acetate	Chloroform	Ethanol	Aqeous
1	Carbohydrates	-	-	+	+	-
2	Glycosides	-	+	-	-	-
3	Saponins	-	-	-	-	+
4	Alkaloids	+	-	+	-	-
5	Flavonoids	-	+	-	+	+
6	Tannins	-	-	-	+	+
7	Proteins	-	-	-	-	-
8	Fixed oils	-	-	-	-	-
9	Steroids	-	+	-	-	-

+ : Presence , - : Absence

Determination of *In vitro* cytotoxic activity of ethanolic and aqueous extracts of whole plant *Pajanelia longifolia* (Willd) K. Schum

The in vitro cytotoxic activity by MTT assay on Hacat(skin cancer cells) and MCF-7(breast cancer cells) were performed. Control, ethanolic and aqueous extracts of *Pajanelia longifolia* (willd) K.Schum were used.

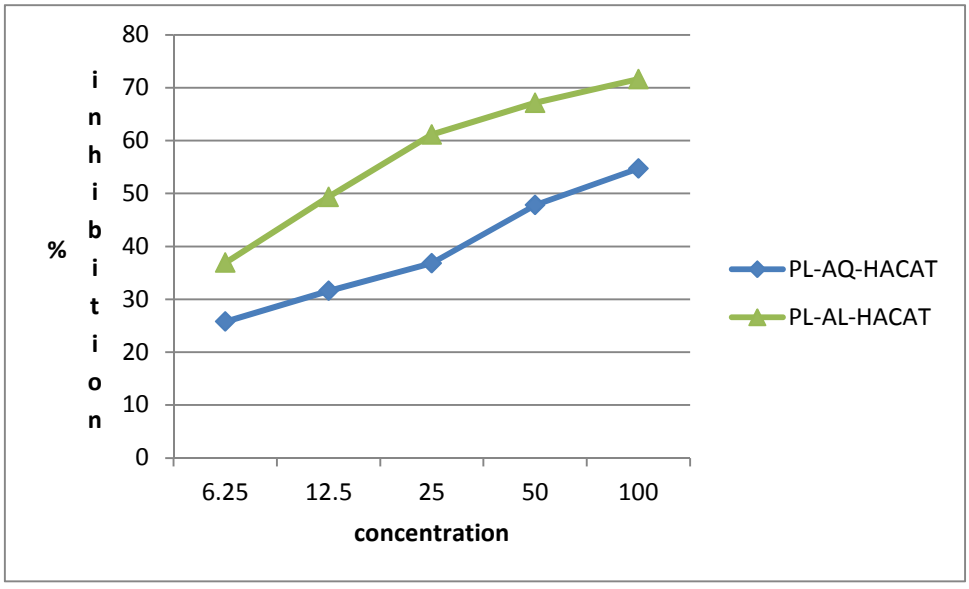
Table 2 % inhibition produced by aqueous extracts of *Pajanelia longifolia* (Willd) K.Schum against HACAT cell lines

Concentration *P.longifolia*., (µg/ml)	Absorbance, Mean ± SD	% inhibition	IC50, µg/ml
6.25	0.3055 ± 0.06	25.78	
12.5	0.2816 ± 0.02	31.58	
25	0.2601 ± 0.03	36.81	
50	0.2149 ± 0.03	47.79	
100	0.1865 ± 0.01	54.69	74.92

Table 3 % inhibition produced by alcoholic extracts of *Pajanelia longifolia* (Willd) K.Schum against HACAT cell lines

Concentration *P.longifolia*., (µg/ml)	Absorbance, Mean ± SD	% inhibition	IC50, µg/ml
6.25	0.2597 ± 0.04	36.90	
12.5	0.2085 ± 0.02	49.34	
25	0.1599 ± 0.02	61.15	15.48
50	0.1353 ± 0.01	67.13	
100	0.11692 ± 0.04	71.59	

Figure 1 Comparison of %inhibition of extracts of of *Pajanelia longifolia* (Willd) K.Schum



MTT Assay using Hacat Cell lines

The results for cell growth inhibition by various extracts against Hacat cell lines for various concentrations is shown in Table 2 & 3 respectively and graphically represented in figure 1. MTT assay of ethanolic and aqueous extracts of *Pajanelia longifolia* (Willd) K.Schum reveals that the ethanolic extract shows more antiproliferative activity against

Hacat cell lines. As the concentration increases there is an increase in the cell growth inhibition, with a maximum IC 50 of 15.48 µg/ml for ethanolic extract.

MTT Assay using MCF-7 Cell lines

Results of different concentrations of ethanolic and aqueous extract of *Pajanelia longifolia* (willd.K.Schum) from 6.25 µg /ml – 100 µg /ml were tabulated in Table 4& 5 respectively and graphically represented in figure 2. MTT assay shows moderate activity for both aqueous and ethanolic extract against breast cancer cell lines. Comparing both extracts aqueous extract shows slight increase in cytotoxic activity with an IC 50 value of 76.62µg /ml. As the concentration increases the percentage inhibition also increased.

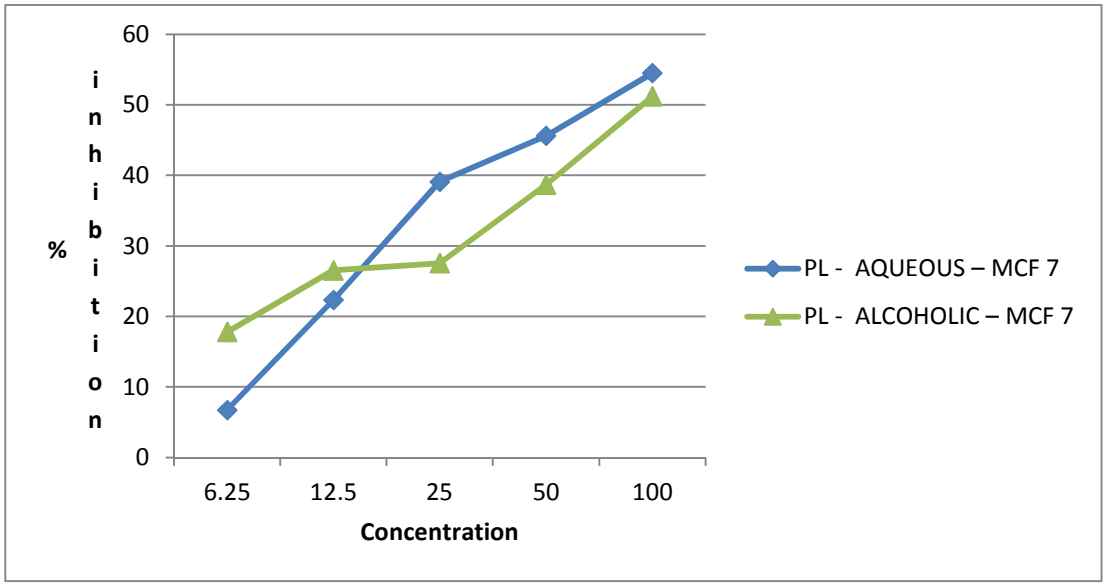
Table 4 % inhibition produced by aqueous extracts of *Pajanelia longifolia* (Willd) *K.Schum* against MCF-7 cell lines

Concentration *P.longifolia.*, (µg/ml)	Absorbance, Mean ± SD	% inhibition	IC50, µg/ml
6.25	0.3595 ± 0.02	6.74	
12.5	0.2994 ± 0.04	22.33	
25	0.2348 ± 0.01	39.09	
50	0.2097 ± 0.01	45.60	
100	0.1754 ± 0.01	54.50	76.62

Table 5 % inhibition produced by alcoholic extracts of *Pajanelia longifolia* (Willd) K.Schum against MCF-7 cell lines

Concentration *P.longifolia.*, (µg/ml)	Absorbance, Mean ± SD	% inhibition	IC50, µg/ml
6.25	0.317 ± 0.01	17.77	
12.5	0.2833 ± 0.01	26.51	
25	0.2793 ± 0.01	27.55	
50	0.2364 ± 0.03	38.68	
100	0.1982 ± 0.01	51.18	98.83

Figure 2 Comparison of % inhibition of extracts of *Pajanelia longifolia (Willd)K.Schum* by *MTT Assay*



CONCLUSION

The current finding concludes *Pajanelia longifolia (Willd) K.Schum* have huge potential to treat cancer. In Present study the ethanolic as well as aqueous extracts shows cytotoxic activity against both Hacat and MCF-7 cell lines, while, ethanolic extract shows better activity against Hacat and aqueous extract shows moderate activity against MCF-7 cell lines. The results are very promising but further studies are required for the isolation and identification of biologically active substances.

REFERENCES

[1] G. Kiranmayi, G. Ramakrishnan, R. Kothai, B. Jaykar,.*Int. j. PharmTech. Res*., **2011**, 33,1329-1333.
[2] W. C. Evans, G. E. Trease., Pharmacognosy, Sanders, London, **2002**, 14, 409.
[3] B. Abhishek, S. Rohini, N. N. Malleshappa., *Int. J. Phy.Med*., **2011**, 3, 9-26.

[4] www.flowersofindia.net/catalog/slides/pajanelia.,2012.
[5] T. Mosman, *J. Immuno. Meth*., **1983**, 65, 55-63.
[6] M. Niks, M. Otto, , *J. Immuno. Meth.*, **1990**, 130, 149-151.
[7] B. P. Minu, T. Sivakumar, P. Giriraj., *Euro. J. Biomed. Pharm.Scies*., **2014**, 3, 542-550.
[8] Dr. A. K. Seth., Pharmaceutics – I, S. Vikas, Jalandhar, **2007**, 2, 204.
[9] T. Mukul, S. Ashok., Phytochemistry, Theory & Practical, Birla Publications, Delhi, **2008**, 1, 133-135.
[10] K. R. Khandelwal., Practical Pharmacognosy – Tech. Exp., Nirali Prakashan, Pune, **2007**,16, 149-154.