



# Cytotoxicity and Antimicrobial effects of *Pistia stratiotes* Leaves

Jayanthi Abraham\*

Priitha Chakraborty

Anish Mathew Chacko

Kaustubh Khare

Microbial Biotechnology Lab,  
School of Biosciences and  
Technology, VIT University,  
Vellore 632014, Tamil Nadu,  
India.

## Corresponding Authors:

Email:

jayanthi.abraham@gmail.com

## Abstract:

*Pistia stratiotes* belongs to family Araceae. *Pistia stratiotes* leaves are traditionally used against ringworm infection of scalp, boils and syphilitic eruptions. Traditionally, oil extracts of *Pistia stratiotes* are used for treatment of tuberculosis, asthma and dysentery. As the effect of *Pistia stratiotes* extracts on the area of cytotoxicity is unexplored, the present work was aimed at evaluating the effect of extracts on cancer cell line and also on clinical pathogens. Phytochemical study was done with methanol extract of *Pistia stratiotes* leaves. Analytical study (GC-MS and TLC) was done with methanol, chloroform and hexane extracts of *Pistia stratiotes* leaves. Antimicrobial activity by well diffusion method and antioxidant activity by DPPH assay were also done. Antimicrobial study was done against eight clinical pathogens namely, *Proteus mirabilis*, *Enterococcus* sp., *Salmonella* sp., *Shigella* sp., *Staphylococcus aureus*, *Serratia* sp., *Klebsiella* sp., *Pseudomonas aeruginosa*. As bone cancer is an aggressive form of cancer MG63 cell line of osteosarcoma was chosen for the study.

**Keywords:** Araceae, antimicrobial activity, antioxidant activity, osteosarcoma, MG 63 cell line, anticancer activity, pathogens, TLC, Gas Chromatography, Mass Spectroscopy.

## Introduction

Herbal plants are being used as medicine from ancient age and usefulness of them are recorded in human history. Herbal plants are reported to be excellent source of several nutrients <sup>(1)</sup>. The use of herbal drugs in treatment of diseases is found among all sections of people in India.

The plant *Pistia stratiotes*, commonly known as water cabbage or water lettuce, belongs to the family Araceae, is an edible, aquatic, floating ornamental plant with widely distributed across tropical and sub-tropical areas around the world. The plant leaves are light green, obovate with prominent longitudinal veins at its base <sup>(2)</sup>. *P. stratiotes* is widely distributed and is being loathed in Asia and Africa. Many organic compounds, metabolites and pigments like vicenin and lucenin have been isolated from *P. stratiotes* <sup>(3)</sup>. This plant and its extracts are potentially believed to have

medicinal effects. *P. stratiotes* leaves are traditionally used against ringworm infection of scalp, boils and syphilitic eruptions. Traditionally, oil extracts of this plant are used for treatment of tuberculosis, asthma and dysentery <sup>(4)</sup>.

Water lettuce is proved to be superior to most other plants in nutrient removal efficiency in the environmental cleanup, owing to its rapid growth and high biomass yield potential <sup>(5)</sup>. Water lettuce appears to be one of the most effective aquatic plants for the bioremediation of mercury and cadmium <sup>(6)</sup>. This plant is proven to be antiseptic, antitubercular and antidysentric. In various parts of the world it is also used as anodyne for eyewash. The leaves are used in eczema, leprosy, ulcers and piles <sup>(4)</sup>. The plant is bitter, pungent flavor, having cooling, laxative property. It is used in 'Tridosha' fever and diseases of blood. The root is laxative, emollient and has diuretic effect. Leaf

infusions have been mentioned in the folklore to be used for dropsy, bladder complaints, kidney afflictions, hematuria, dysentery and anemia <sup>(7)</sup>.

The studies over antioxidant effects on the cancer cell line cultures are not much established. This study is undertaken to evaluate the antimicrobial, anticancer and antioxidant scavenging effects of *Pistia stratiotes* leaf extracts by using experimental approach.

## Materials and methods

### Chemicals

All chemicals and solvents used in this study were of high purity and analytical grade.

### Sample extraction

The *Pistia stratiotes* leaves were collected dried and powdered. Powdered sample were extracted with methanol, hexane, chloroform using soxhlet apparatus. The extracted solvent is later collected, dried and stored for further studies.

### Phytochemical studies

Phytochemical studies were done with methanol extract of plant leaves to primarily detect the presence of various compounds.

### Detection of alkaloids

Solvent free extract, 5 mg was stirred with few ml of diluted hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents <sup>(8)</sup>.

A. Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids was confirmed by the formation of yellow coloured precipitate.

B. Wagner's Test: To few ml of filtrate, few drops of Wagner's reagent were added along the side of test tube. A reddish brown precipitates indicated positive test. <sup>(9)</sup>.

Wagner's reagent: iodine (1.27g) and potassium iodide (0.92 g) was dissolved in 5 ml of water and made up to 100 ml with distilled water.

### Detection of carbohydrates and glycosides

5 mg of extract was dissolved in 5 ml of water and filtered. The filtrate was subjected to the following tests. <sup>(10)</sup>

A. Fehling's Test: 1 ml of filtrate was boiled on water bath with 1 ml of each of Fehling's solutions A and B. Appearance of red precipitate confirmed the presence of sugar.

Fehling's solution A: Copper sulphate (34.66 g) was dissolved in distilled water and made up to 500 ml using distilled water.

Fehling's solution B: Potassium sodium tartarate (173 g) and sodium hydroxide (50g) was dissolved in water and made up to 500 ml.

B. Molish Test: to 2 ml of filtrate, two drops of alcoholic solution of a naphthol were added, the mixture was shaken well and 1 ml of conc. sulphuric acid was added slowly along the sides of test tube and allowed to stand. Formation of violet ring indicated the presence of carbohydrates.

### Detection of phytosterols

Liebermann Burchard's Test: The extract (5 mg) was dissolved in 2 ml acetic anhydride. To this, one or two drops of conc. sulphuric acid were added slowly along the sides of the test tube. An array of colour changes indicated the presence of phytosterols <sup>(11)</sup>.

### Detection of phenolic compounds

Ferric chloride test: The extract (2 mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution was added. Appearance of green colour indicates the presence of phenolic compounds <sup>(12)</sup>.

### Detection of flavonoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, which indicated the presence of flavonoids.

Lead acetate Test: To 1ml of the plant extract was added in a test tube. To this 1ml of 5% lead acetate and the mixture was allowed to stand for few minutes. The formation of precipitates in samples confirmed the presence of flavonoids.

### Detection of Tannins

About 0.5 g of extract was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate. Formation of a bluish black, bluish green or green precipitate confirmed the presence of tannins.

### Detection of terpenoids (Salkowski test)

2 ml of chloroform of chloroform was added to the extract. Concentrated H<sub>2</sub>SO<sub>4</sub> (3 ml) was carefully added to form a layer. Appearance of reddish brown colouration on the interface indicates the presence of terpenoids.

## Analytical methods

### Fractionation of the crude extract using TLC

Using pre-coated TLC F254 plates, the crude extract was fractionated using different combinations of hexane/chloroform/methanol solvents (2:1:1) and methanol/hexane (3:2) as the mobile phase. Separated components were viewed in visible light, under UV at 360 nm, by fluorescence quenching less than 254 nm. Separation done with mobile phase of hexane: chloroform: methanol was fractionated and recorded.

### Gas Chromatography Mass Spectrometry

Methanol and hexane extract of *Pistia stratiotes* leaves were analyzed by GC-MS. Perkin Elmer Clarus 680 gas chromatographic instrument equipped with a mass spectrometer detector (Clarus 600 model) and an Elite-5MS (30.0 m, 0.25 mmID, 250 µm df) column was used. The carrier gas used was helium at a flow rate of 1 ml min<sup>-1</sup>. The following temperature program was used: initially the oven temperature was held at 60°C for 2 min and then ramped from 10°C/min to 300°C with hold time for 4 min, total run time 30 min. The temperature of the injector was maintained at 300°C. The ion trap was operated at 70 eV with a scan range of m/z from 50 to 600. A sample of 1 µl was injected in split mode (10:1). The intermediate and end product was identified based on the Wiley registry of mass spectral data.

### Antimicrobial study

The antibacterial activity of three different extracts (methanol, chloroform and hexane) of *Pistia stratiotes* plant leaves against eight bacterial pathogens was evaluated by using agar well diffusion method<sup>(13,14)</sup>. Muller Hinton Agar (MHA) plates were inoculated with selected bacterium. Wells of 8 mm size were made with sterile borer on agar plates. Four different volumes (25 µl, 50µl, 75 µl, 100 µl) of the plant extract were poured into each well of inoculated plates. Respective solvent for particular solvent extracts was used as a negative control. Then they were left at room temperature for ten minutes allowing the diffusion of the plant extract into the agar<sup>(15)</sup>. After incubation for 24 hrs at 37°C, the plates were observed for clear zone. Antibacterial activity of the extract was identified by an inhibition zone surrounding the well containing the plant extract. The zone of inhibition was measured and expressed in millimeters (mm). Clear zone of the

plant extract and comparison with negative control was recorded <sup>(16)</sup>.

### Antioxidant Study

The antioxidant activity of the methanol, chloroform and hexane extracts was evaluated by DPPH radical scavenging assay which was originally described by Blois <sup>(17)</sup>. DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a synthetic free radical with deep violet colour when is in form of solution which has a  $\lambda_{max}$  at 517nm. It can accept an electron or hydrogen radical to become stable diamagnetic molecule and appear as light purple in colour which indicates the scavenging of DPPH and the substance has antioxidant activity.

Methanol solutions were prepared with all the three extracts. Methanol solution of DPPH was used as negative control. 500 $\mu$ l of each sample and 500  $\mu$ l of DPPH solution was allowed to react and incubated at room temperature for 30mins under dark conditions. Absorbance was taken at  $\lambda_{max}$  i.e. 517nm against a blank which was 500 $\mu$ l of methanol. Percentage inhibition was calculated by the following equation to conclude the presence of antioxidant activity of the extracts.

. Percentage of inhibition = (OD control – OD sample / OD control) x 100

### Anticancer study

#### Cell line:

The human osteosarcoma cell line (MG 63) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37°C, 5% CO<sub>2</sub>, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

#### Cell treatment procedure:

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1x10<sup>5</sup> cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO<sub>2</sub>, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100  $\mu$ l of these different sample dilutions were added to the appropriate wells already containing 100  $\mu$ l of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37°C, 5% CO<sub>2</sub>, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

#### MTT assay:

After 48 h of incubation, 15 $\mu$ l of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 $\mu$ l of DMSO and then measured the absorbance at 570 nm using micro plate reader.

The percentage of cell viability was then calculated with respect to control as follows

Percentage of cell viability = (A) Test / (A)control x 100

The percentage of cell inhibition was determined using the following formula.

$$\text{Percentage of cell inhibition} = 100 - \frac{\text{Abs (sample)}}{\text{Abs (control)}} \times 100.$$

Nonlinear regression graph was plotted between percentage of cell inhibition and Log concentration and IC<sub>50</sub> was determined using GraphPad Prism software.

## Results

### Phytochemical study

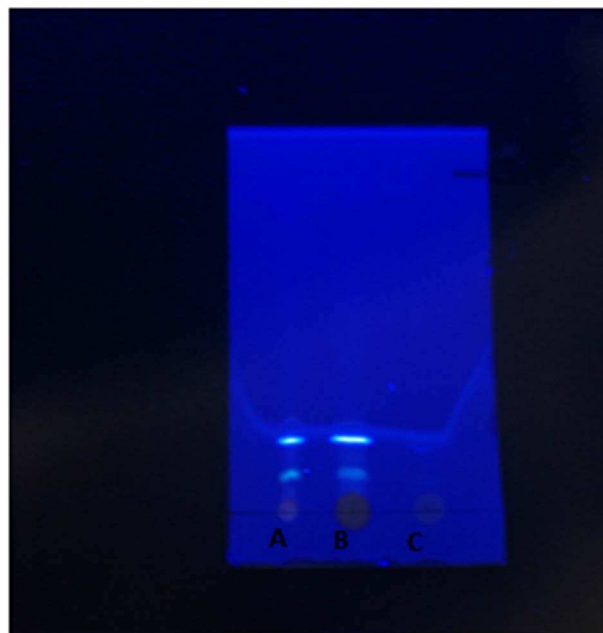
In recent years, secondary plant metabolites extensively investigated as a source of medicinal agents. It has been accepted that natural compounds play an important role in health care. Water cabbage leaves are source of various bioactive compounds. Result of phytochemical studies of plant extract with methanol is presented in a table form in table 1. According to the result, alkaloids, phytosterols, phenols, flavonoids and tannins are present in *Pistia stratiotes* leaves. However Fehling's and Molish test for carbohydrate and Salkowski test for terpenoids showed negative response. According to Singh et al. (18) phenolic compounds possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, as well as inhibition of angiogenesis and cell proliferation activities. Phytosterol acts as growth hormones in plants. The plant has medicinal property due to presence of these phytochemicals (19).

### Analytical studies

#### TLC

TLC was done to fractionate each components of the extract by its characteristic R<sub>f</sub> values. Separated spots were observed under UV light (fig.1). Two separated spots have been observed under UV light for methanol and chloroform

extract of *Pistia stratiotes* leaves. But no separation was observed for hexane extract. Separated spots on TLC plates indicate presence of different compounds which were further analyzed by GC-MS.



**Fig 1:** Separated spots on TLC plates visualized under UV light; A: methanol extract, B: chloroform extract, C: hexane extract.

#### GC-MS

In order to determine the compounds present in the extract of *Pistia stratiotes* leaves, GC-MS analysis was done. This analysis revealed that methanol, chloroform and hexane extract of *P. stratiotes* leaves contain different compounds. Some of them are known for their biological activity whereas activity of a few compounds remains unknown. The GC-MS chromatogram of methanol, chloroform and hexane extracts are presented in fig 2, fig 3, and fig 4 respectively. From GC-MS analysis, it has been seen that methyl 17-methyl-octadecanoate is present in chloroform extract of *P. stratiotes* leaves, which is reported to possess antimicrobial, antioxidant and antitumor activity (20). N-hexadecanoic acid (palmitic acid), found in methanol and hexane extract of *Pistia*



*stratiotes* leaves which possess antibacterial and cholesterolaemic effects, selective toxicity to human leukemic cells. It also has shown in vivo antitumor activity in mice by making a target to DNA topoisomerase I<sup>(21,22)</sup>. Whereas activity of Z,Z-6,28-hepatatriactontadien-2-one present in both methanol and hexane extract remained unknown. Activities of these compounds are believed to be responsible for the activities shown by *P. stratiotes* leaves extracts.

#### Antimicrobial Test

Antimicrobial activity of *P. stratiotes* leaves was checked against eight clinical pathogens. Antimicrobial activity was determined by measuring zone of inhibition formed after incubation period. No inhibition zone was observed for chloroform and hexane extracts against eight test pathogens. The methanol extract showed characteristic zone of inhibition against five pathogens including *Pseudomonas aeruginosa*, *Shigella* sp., *Serratia* sp., *Salmonella* sp. and *Klebsiella* sp. among eight test pathogens. The zone of inhibition was very pronounced. Highest zone of inhibition was observed at 100mg/ml concentration against *Klebsiella* sp. Methanol extract of *P. stratiotes* leaves showed antimicrobial activity against both Gram positive and Gram negative<sup>(23)</sup>. Table 2 indicates the result of the antimicrobial activities of methanol extract of *P. stratiotes* leaves.

#### Antioxidant Test

The antioxidant activity of the extracts was evaluated by DPPH radical scavenging assay which was originally described by Blois<sup>(17)</sup>. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a synthetic free radical with deep violet colour when is in form of solution which has a  $\lambda_{max}$  at 517nm. Among methanol, hexane and chloroform extract of *Pistia stratiotes* leaves, methanol extract has shown

highest antioxidant activity where hexane and chloroform extract has shown moderate activity (table 3). The reducing power of methanol extract indicates presence of some compounds in *Pistia stratiotes* extracts which can donate electron and could react with free radicals to convert them into more stable products and to terminate radical chain reactions. Increased absorbance of reaction mixture indicates increased reducing power of the extract.<sup>(24)</sup>

#### Anticancer study

Anticancer study of the *Pistia stratiotes* leaves was done against human osteosarcoma cell line (MG 63). Anticancer activity of methanol and chloroform extract was checked. The activity of methanol and chloroform extract was shown in fig 5 and fig 6 respectively. In methanol extract cell viability is decreased with increased concentration of extract, which indicates the moderate activity of the extract. On the other hand, 1.0  $\mu$ g/ml concentration of extract showed less than 20% of cell inhibition while at 2.5  $\mu$ g/ml concentration of extract, percentage of cell inhibition reaches 60%, which indicates that percentage of cell inhibition is increased with increasing concentration of chloroform extract<sup>(25)</sup>. It indicates the presence of anticancer activity of both methanol and hexane extract of *P. stratiotes* leaves.

## Discussion

From the above study, the plant *Pistia stratiotes*, is proven to have good antimicrobial, antioxidant and anticancer activities. As the above result are very promising and encouraging to continue research on *Pistia stratiotes* leaves and there is a need to purify the compounds to enhance their

activity and make them suitable for further clinical approach in near future.

**Table 1:** Phytochemical properties of methanol extract of *Pistia stratiotes* leaves.

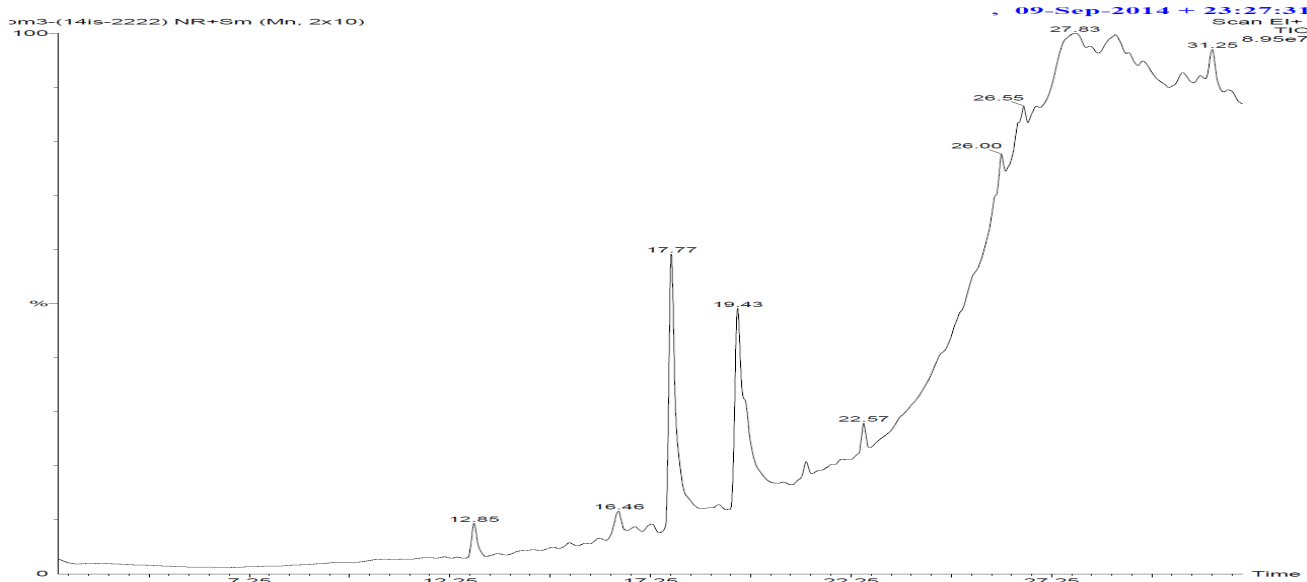
Sl. No.	Phytochemical test	Result
1	Alkaloids	
	Hager's test	+
	Wagner's test	+
2	Carbohydrates	
	Fehling's test	-
	Molish Test	-
3	Phytosterols	
	Liebermann Burchard's Test	+
4	Phenols	
	Ferric chloride test	+
5	Flavonoids	
	Alkaline Reagent Test	-
	Lead acetate Test:	+
6	Tannin	+
	Terpenoids	
7	Salkowski test	-

**Table 2:** Zone of inhibition of methanol extract of *Pistia stratiotes* leaves against eight pathogens.

Sl no	Organisms	Zone of inhibition (cm)			
		25mg/ml	50mg/ml	75mg/ml	100mg/ml
1	<i>Pseudomonas aeruginosa</i>	0.9	1.0	1.2	1.3
2	<i>Shigella</i> sp.	-	0.7	1.0	1.3
3	<i>Serratia</i> sp.	-	1.0	1.3	1.5
4	<i>Salmonella</i> sp.	-	-	1.0	1.3
5	<i>Klebsiella</i> sp.	1.0	1.2	1.3	1.5
6	<i>Enterobacter</i> sp.	-	-	-	-
7	<i>Proteus mirabilis</i>	-	-	-	-
8	<i>Staphylococcus</i> sp.	-	-	-	-

**Table 3:** Percentage of inhibition of different extracts of *Pistia stratiotes* leaves.

Sample	OD Sample At 517nm	Percentage Of Inhibition
Standard	0.823	
Methanol extract	0.445	45%
Chloroform extract	0.483	34%
Hexane extract	0.740	10%



**Fig. 2:** GC-MS chromatogram of *Pistia stratiotes* leaves methanol extract.

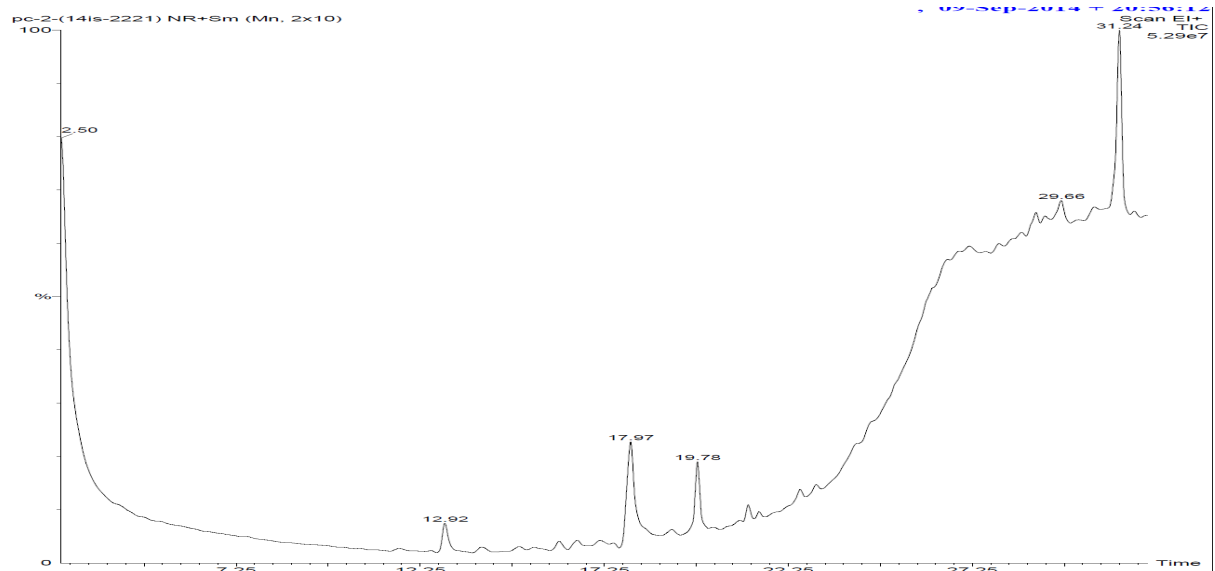


Fig 3: GC-MS chromatogram of *Pistia stratiotes* leaves chloroform extract.

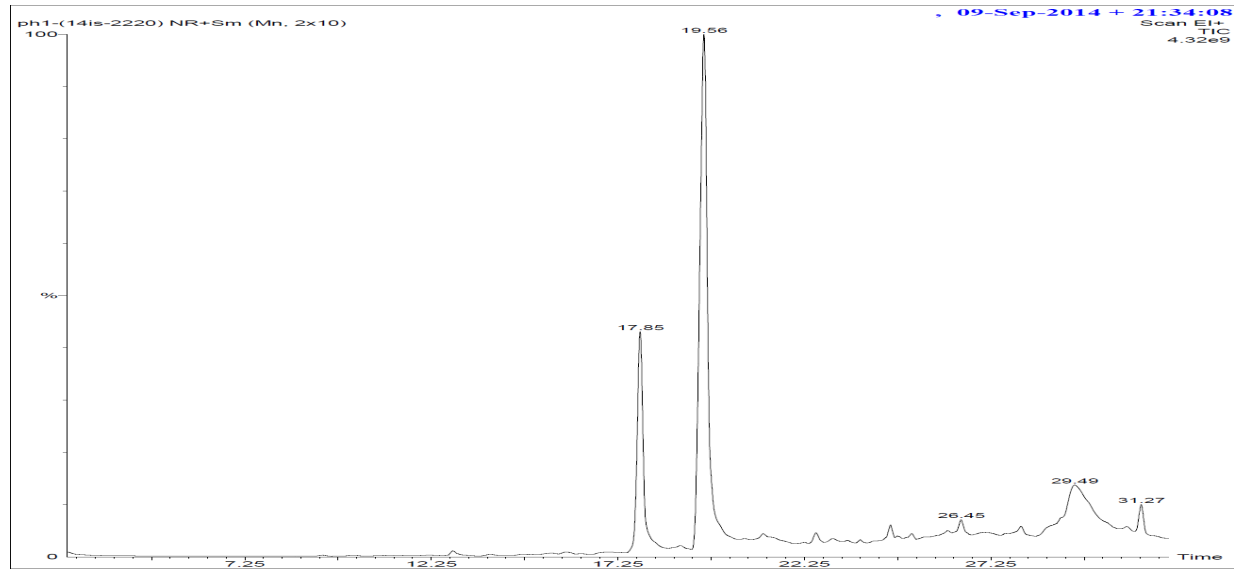


Fig 4: GC-MS chromatogram of *Pistia stratiotes* leaves hexane extract.

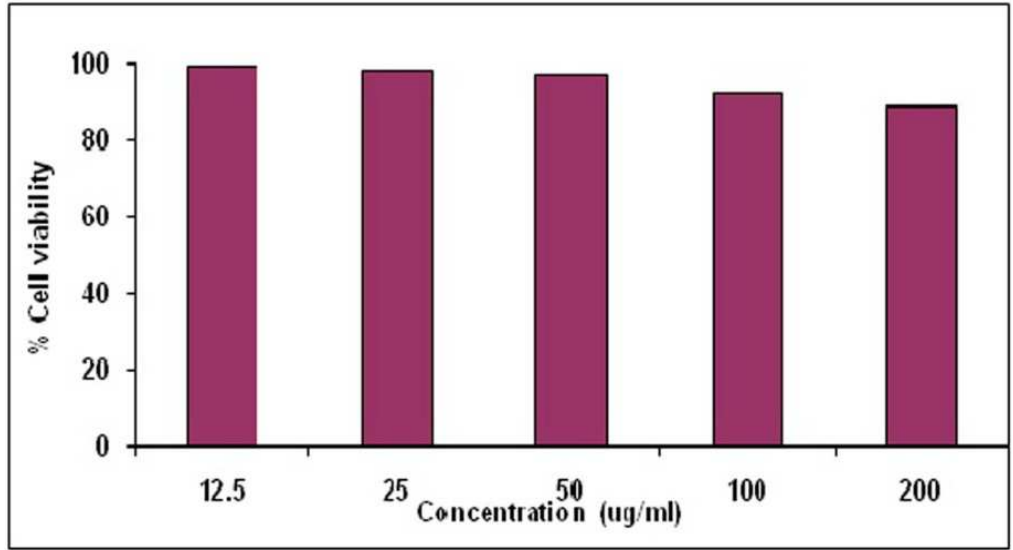


Fig 5: Anticancer activity of methanol extract of *Pistia stratiotes* leaves



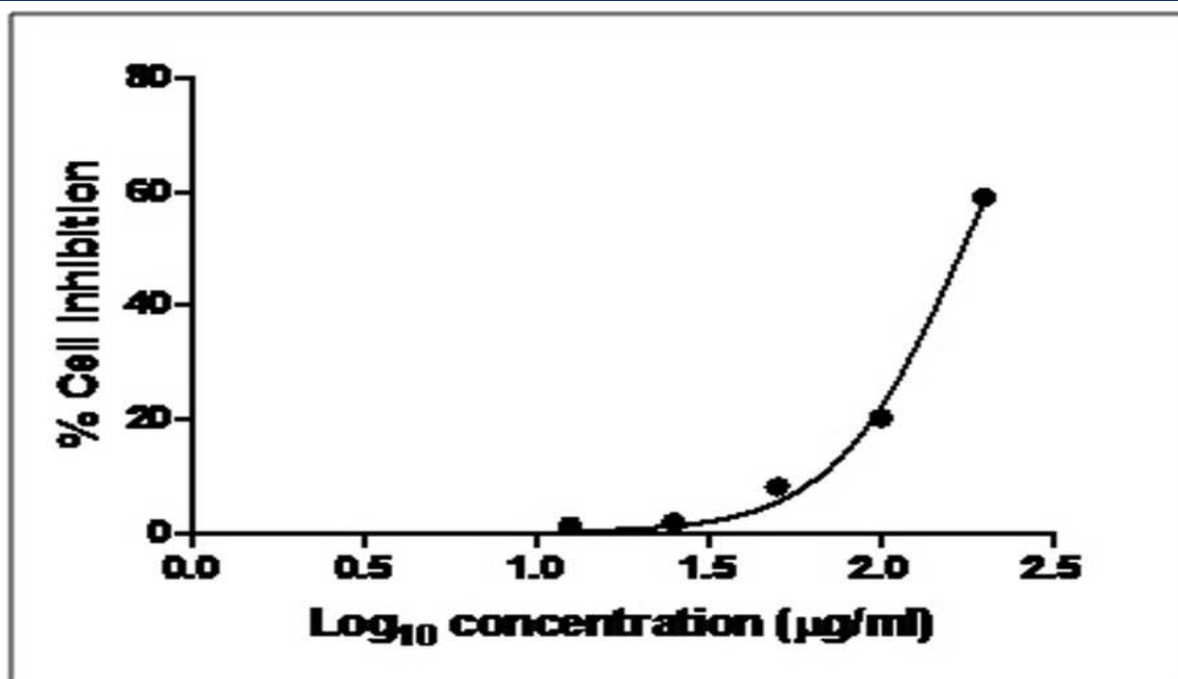


Fig 6: Anticancer activity of chloroform extract of *Pistia stratiotes* leaves

## References

- Musa O. Mineral composition of different parts of *Capparis onila* Dest Var. *Cenescens* (Coss) Heywood growing wild in Turkey. *Journal of Medical Food*, 2005;8: 405-407.
- Arber A. The vegetative morphology of *Pistia* and the Lemnaceae. *Proc Roy Soc*, 1991; 91:96-103.
- Marina DG, Antonio M, Pietro M, Lucio P. Acylglycosyl sterols from *Pistia stratiotes*. *Phytochemistry* 1991;30(7):2422-2424.
- Kirtikar KR, Basu BD. *Indian Medicinal Plants*. Delhi: Sri Satguru Publications. 2000.
- Qin Lu & Zhenli L. He & Donald A. Graetz, Peter J. Stoffella & Xiaoe Yang. Phytoremediation to remove nutrients and improve eutrophic stormwaters using water lettuce (*Pistia stratiotes* L.). *Environ Sci Pollut Res* 2010; 17:84-96.
- Skinner, K., Wright, N., Porter-Goff, E. Mercury uptake and accumulation by four species of aquatic plants. *Environ. Pollut.* 2007;145:234-237.
- Kirtikar, K.K. and B.D. Basu. *The Indian medicinal plants*. Dehradun: Oriental Enterprises. 2001. 3576-3579.
- Evans WC. 1997. *Pharmacology*. Harcourt Brace and Company. Asia, Singapore. 226.
- Wagner H. 1993. *Pharmazeutische Biologie* (5th Ed.) AUFI. 15 BN 3- 437-20 498-X. Gustav fisher Vwlag. Stuttgart.Germany. 184.
- Ramakrishna et al, *From Physiology and Chemistry to Biochemistry*. *Theor Applied Genet* 1994; 88: 402
- Finar IL. 1986. *Stereo chemistry and chemistry of natural products*. 2: Longman, Singapur. 682
- Mace ME.. *Histochemical localization of phenols in healthy and diseased banar roots*. *Physiol plant*. 1963; 16: 915-925.
- Ahmad I, Beg AZ. *Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens*. *J Ethnopharmacol*. 2001;74(2):113-23.
- Srinivasan, D., S. Nathan, T. Suresh and O. Perumalsamy. *Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine*. *J. Ethnopharm.* 2001; 74:217-220.
- Rios JL, Recio MC, Villar A. *Screening methods for natural products with antimicrobial activity: a review of the literature*. *J Ethnopharmacol*. 1988;23(2-3):127-149.
- Hammer KA, Carson CF, Riley TV *Antimicrobial activity of essential oils and other plant extracts*.

- Journal of Applied Microbiology 1999; 86: 985-990.
- 17) Blois MS, Zhao XY. Antioxidant determinations by the use of a stable free radical, Nature. 1958; 181: 1199-1200.
  - 18) Singh R, Singh SK, Arora S. Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A. Cunn. Food Chem. Toxicol. 2007; 45: 1216-1223.
  - 19) Dethe UL, Joshi SS, Desai SS, and Aparadh VT. Screening of bioactive compounds of *Sesbania grandiflora* and *Pistia stratiotes*, Indian Journal of Advances In plant Research 2014; 1(1); 27-30
  - 20) G. Agoramoorthy, M. Chandrasekaran, V. Venkatesalu, MJ Hsu. Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India, Braz. J. Microbiol. 2007; 38(4) :739-742.
  - 21) Harada, H., U. Yamashita, H. Kurilara, F. Fukushi, J. Kawabata and Y. Kamei, 2002. Antitumor activity of palmitic acid found as a selective cytotoxic substance in marine red algae. Anticancer Res., 22: 2587-2590.
  - 22) Saxena, M., U. Faridi, S.K. Srivastava, M.P. Darokar, R. Mishra, A. Pal, B. Shisodia and S.P.S. Khanuja, 2007. A cytotoxic and hepatoprotective agent from *Withania somnifera* and biological evaluation of its ester derivatives. Nat. Prod. Communic., 2:1-4.
  - 23) Harami M. Adamu, OJ. Abayeha, MO. Aghoa, AL. Abdullahi, A. Uba, HU. Dukku, BM. Wufem. An ethnobotanical survey of Bauchi State herbal plants and their antimicrobial activity. Journal of Ethnopharmacology 2005; 99: 1-4.
  - 24) Megha Jha, N. Ganesh, and Versha Sharma. In vitro Evaluation of Free Radical Scavenging Activity of *Pistia stratiotes*. International Journal of Chem Tech Research 2010; 2: 180-184.
  - 25) Letícia Veras Costa-Lotufo, Mahmud Tareq Hassan Khan, Arjumand Ather, Diego Veras Wilke, Paula Christine Jimenez, Cláudia Pessoa, Maria Elisabete Amaral de Moraes, Manoel Odorico de Moraes. Studies of the anticancer

potential of plants used in Bangladeshi folk medicine. Journal of Ethnopharmacology 2005;99: 21-30.

**Article History:** .....

Date of Submission: 16-12-2014

Date of Acceptance: 29-12-2014

Conflict of Interest: NIL

Source of Support: NONE

