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Research Article

ANTIPROLIFERATIVE ACTIVITY OF ETHANOLIC FLOWER EXTRACT FROM *NYMPHAEA PUBESCENS* WILLD AGAINST HUMAN CERVICAL AND BREAST CARCINOMA *IN VITRO*

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ABSTRACT
Nymphaea pubescens Willd (Nymphaeaceae) is a fascinating aquatic plant mentioned in siddha system of medicine, in the treatment of bleeding piles, diabetes and as cardiotonic in palpitation of the heart. *Nymphaea* species was traditionally used for treating cancer. The present study was designed to evaluate the invitro antiproliferative activity of *Nymphaea pubescens* Willd. The ethanolic extract of different parts such as rhizome, leaf, flower and fruit was subjected for MTT assay. The ethanolic extract of flower part was found to be cytotoxic against human cervical carcinoma Hela cell lines and human breast carcinoma MCF cell lines. The IC50 value of ethanolic flower extract was 91.57µg/ml against Hela cell lines and 99.6µg/ml against MCF-7 cell lines. Significant results were observed thereby justifying the use of plant in the traditional system of medicine
Keywords: MTT assay, Antiproliferative activity, *Nymphaea pubescens*, cervical carcinoma, Breast carcinoma

INTRODUCTION

Plant derived natural products such as flavonoids, terpenes, alkaloids and so on have received considerable attention in recent years due to their diverse pharmacological properties, including cytotoxic and cancer chemoprotective effects[1]. Over 50% of the drugs in clinical trials for antitumor activity were isolated from natural source or are related to them[2]. Several plant products have been tested for antitumor activity and some of these, such as vincristine and taxol are now available as drugs of choice[3]. One of the best approaches in the search for antitumour agents from plant resources is the selection of plant based on ethnomedical leads and testing the selected plants efficacy and safety through modern scientific methods[4].

Nymphaea pubescens Willd (Nymphaeaceae) is a perennial aquatic rhizomatous stoloniferous herb. It is commonly known as water lily, which includes about fifty species and widely distributed in tropical and temperate regions, inhabiting stagnant fresh water, ponds, lakes and swamps. The medico ethnobotanical review of the flower of *Nymphaea pubescens* was used as blood purifier and in the treatment of jaundice[5]. *Nymphaea* species were used in the treatment of diabetes, cancer, inflammation and eyedisorder[6]. There is no scientific literature for antiproliferative activity of *Nymphaea pubescens* and hence the study was designed to investigate the antiproliferative activity of ethanolic extract from different parts such as rhizome, leaf, flower and fruit of *Nymphaea pubescens* by MTT cell proliferation assay.

MATERIALS AND METHODS

Plant material & Extraction

The plant material were collected in the ponds of Oomangalam village in Neyveli, Tamilnadu, India, in the month of march 2011 and it was botanically identified and authenticated by Prof. Jayaraman, Plant Anatomy Research Centre, Thambaram, Chennai, Tamilnadu, India. A voucher specimen PARC/2007/79 was deposited at the Department of Pharmacognosy, college of Pharmacy, Madras Medical college, Chennai, Tamilnadu, India. The shade dried different parts such as Rhizome, leaves, flower and fruit of *Nymphaea pubescens* was coarsely powdered and extracted with ethanol using soxhlet extraction apparatus until exhaustive extraction. The solvent was removed using rotary vacuum evaporator and solvent free extract were subjected for MTT cell proliferation assay[7].

MTT cell proliferation assay

Cell line and culture

The cell line of Hela (Human cervical carcinoma), MCF-7 (human breast carcinoma) were obtained from National Centre for Cell Science, Pune, India. The cells were cultured in a growth medium (DMEM, PH-7.4), supplemented with 10% fetal bovine serum (FBS) and antibiotics, Penicillin (100 units/ml) and streptomycine sulfate (100µg/ml)[8].

MTT assay

The cells were seeded into wells of a 96 well microtiter plate (Costar 3599, corning, NY, USA) at 2 x 10^4 cells per well with 100 µl, DMEM growth medium and then incubated for 24 hours at 37°c under 5% CO_2 in a humidified atmosphere. Later, the medium was removed while fresh growth medium containing different test dose at 100, 50, 25, 12.5, 6.25, 3.125µg/ml were added. After 3 days of incubation at 37°c under 5%CO_2, the medium was removed before adding 100µl DMSO to each well and gently shaken. The absorbance was then determined by ELISA reador (Biorad, Mercules, California, USA) at 490nm. Control wells received only the media without the test sample. The conventional anticancer drug, 5-fluorouracil[9], was used as a positive control in this study. The inhibition of cell growth was calculated as a percent antiproliferative activity using the following formula

$$\text{Cells inhibition} = \frac{\text{Control absorbance-sample absorbance}}{\text{Control absorbance}} \times 100$$

Statistical analysis

Data were expressed as Means ± standard deviations of three replicate determinations and then analyzed by SPSS v.13 one way analysis of variance (ANOVA) and dancan's new multiple range test were used to determine the differences among the means. P values<0.05 were regarded as significant[10].

RESULTS AND DISCUSSION

The MTT assay is based on the reduction of MTT (3-(4,5-dimethyl thiazolyl)-2,5-diphenyl-tetrazolium bromide) by mitochondrial dehydrogenase to purple formazan product. The ethanolic extract from different parts such as rhizome, leaves, flower and fruit were subjected for MTT cell proliferation assay and results are presented in table.1. Among different parts the ethanolic flower extract was found to have cytotoxic activity although only extract with an IC_{50} value lower than 200µg/ml were considered active (Kviecinskie et al.,2008). The other extracts were examined and the IC_{50} value shows higher than 200µg/ml was considered inactive[11]. The photograph of ethanolic flower extract shows the apoptosis human cervical carcinoma Hela cell lines (Fig.1) and human breast carcinoma MCF-7 cell lines (Fig.2).

CONCLUSION

The MTT assay of ethanolic extract from different parts of *Nymphaea pubescens* Willd led to the identification of considerably potent ethanolic flower extract. This extract was able to induce apoptosis on human cancer cell lines and its antiproliferative activity was found to be specific. Further work is required in order to establish the identity of the chemical constituent responsible for antiproliferative activity. Studies are in progress on our laboratory to elucidate the molecular and cellular mechanism of the ethanolic flower extract *in vivo* which contribute towards the development of potent antiproliferative drug.

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Table 1: The IC_{50} values of ethanolic flower extract from *Nymphaea pubescens* against human cervical carcinoma Hela and human breast carcinoma MCF-7 cell lines

Parts Ethanol extract	Cytotoxicity IC_{50} values	
	Hela	MCF
Rhizome	318.3	310.5
Leaves	315.8	320.7
Flower	91.57	99.6
Fruit	408.5	416.3
5-Fluoro uracil	19.6	5.3

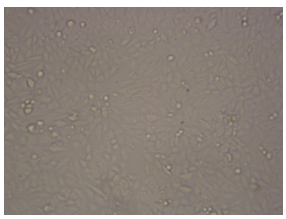

Fig.1 Microscopic observation of Hela cells undergoing apoptosis treated with ethanolic flower extract for 48 hours (Original magnification 320x)

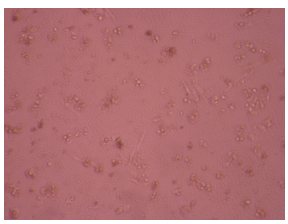

Fig 2 Microscopic observation of MCF cells undergoing apoptosis treated with ethanolic flower extract for 48 hours (Original magnification 320x)

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