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Research Article

Assessment of *in vitro* antioxidant and cytotoxic potentials of ethanol extract of *Nymphaea nauchali*

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Abstract

Objective: The present study was designed to evaluate ethanol extract of *Nymphaea nauchali* for anticancer against breast cancer cells and also to determine its antioxidant properties.

Methods: The ethanol extract of *Nymphaea nauchali* was prepared using sox-let apparatus. The ethanol extract was evaluated for its in-vitro anticancer potentials against human MCF-7 cancer cell lines by MTT assay and concentration of the ethanol extract required to inhibit 50% of cell growth (IC_{50}) was recorded. To determine antioxidant properties of the extract, Superoxide scavenging, Lipid peroxidation and DPPH methods were used and IC_{50} values of extract was noted.

Results: In the present study, ethanol extract of *Nymphaea nauchali* has shown anticancer property by exhibiting significant IC_{50} values against MCF 7 cells. The extract has also shown significant IC_{50} values in Superoxide scavenging, Lipid peroxidation and DPPH methods and antioxidant activity was comparable to standard drug ascorbic acid.

Conclusion: The results obtained from the present research work suggested that the ethanol extract of *Nymphaea nauchali* possess significant *in vitro* cytotoxic potentials against breast *cancer* cells. The results also suggested that the ethanol extract have *in vitro* antioxidant properties.

Keywords: Anticancer activity, *Nymphaea nauchali,* IC_{50} value, MTT assay and antioxidant activity.

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INTRODUCTION

Cancer is serious and common disease condition which kills peoples in the society more than infectious diseases such as tuberculosis, malaria and HIV/AIDS combined[1]. Cancer has become major health problem worldwide and claims more than 10 million people dies a year due to breast, lung, liver and colon ovarian cancers[2]. According to estimation about 12.7 million cancer cases and 7.6 million cancer deaths are reported in 2008 among which 56% of the cases and 64%of the deaths occurred in the economically developing world[3]. On the Indian scene, about 1.1 million new cancer cases reported per year which stands India as a single country among 184 countries and contributes about 7.8% of deaths to global cancer mortality figures[4]. Cancer has the second highest mortality rate after cardiovascular diseases throughout the world. Even though remarkable progress has been made by medical science, the availability of safe and specific anticancer drugs has remained a major challenge in clinical practice[5,6]. Although the cancer research has led to a number of new and effective solutions, the medicines used as treatments have clear limitations and unfortunately cancer is projected as the primary cause of death in the future[7,8]. Antioxidants are a group of substances that are useful for fighting cancer and other processes that potentially lead to diseases such as atherosclerosis, Alzheimer, Parkinson, diabetes and heart disease. Antioxidants act by preventing the onset of cancer during carcinogenesis and they are generally beneficial to cells[9].

Medicinal plants have been used since ancient time in Ayurveda and other traditional medicine system and their utility has been increasing day by day throughout world. Natural compounds obtained from herbs are considerably safe and effective than synthetic compounds. Moreover, the problem of development of drug less compared to synthetic drugs. is also reduced. The herbal drugs comprise a major source of anticancer medicine due to presence of various phyto-constituents such as vincristine, vinblastine, taxanes, camptothecines and in developed countries and developing countries[9]. The free radicals are the main agents which induces mutation by damaging cellular DNA ultimately leads to development of cancers in the body. Hence much attention has been given on the development of anticancer agents that possess antioxidant property due to their free radical scavenging potential which plays an important role in protection of DNA free radical mediated damage[10].

Nymphaea nouchali also known as *Nymphaea stellata* belongs to family *Nymphaeaceae* is an important and well-known medicinal plant, widely used in the Ayurveda and Siddha systems of medicines for the treatment of diabetes, inflammation, ulcers, liver disorders, urinary disorders, menorrhagia, blenorrhagia, menstruation problem, as an aphrodisiac, and as a bitter tonic[11,12]. Though *Nymphaea*

nouchali widely used for the management of cancer in traditional and folklore medicine but doesn't have the scientific evidence for the same[13]. Hence the present study was designed to assess the *in vitro* anticancer and antioxidant activity of ethanol of *Nymphaea nauchali*.

MATERIALS AND METHODS

Plant material

The areal parts of plant *Nymphaea nauchali* was collected from surroundings of Bangalore and authenticated by Dr. V Rama Rao, Research Officer, Regional Ayurveda Research Institute For Metabolic Disorders, HOD, Bangalore. The specimen herbarium (Ref. No. RRCBI-11329) was preserved at institute herbarium library.

Preparation of ethanol extract

The shade dried plant material was pulverized into powder and passed through sieve No. 22 mesh. About 350 g (appx.) of coarse powder was subjected to successive solvent extraction using petroleum ether and ethanol in soxhlet's apparatus[14]. The percentage yield of ethanol extract was found to be 14.22.

Preliminary phytochemical investigation

The preliminary phytochemical investigation for the ethanolic extract of *Nymphaea nauchali* had been conducted as per procedure prescribed by Khandelwal[15].

Drugs and chemicals

All reagents and chemicals used in the study were obtained commercially and were of analytical grade. The standard drugs tamoxifen and paclitaxel were obtained as gift samples from Strides laboratories, Bangalore.

Evaluation of antioxidant property

The evaluation of antioxidant activity of ethanol extract of *Nymphaea nauchali* was carried out by the following two methods:

Lipid peroxidation method

Lipid peroxidation inhibition was estimated by the formation of colored product in the reaction mixture. The assay mixture contained the ethanol extract in various concentrations (50-400 µg/ml), to which were added 0.1 ml of potassium chloride (30 mM), 0.1 ml of ascorbic acid (0.06 mm) and 0.1 ml of ammonium ferrous sulphate (0.16 mM) in succession. Later the reaction mixture was treated with 0.2 ml of sodium dodecyl sulphate (8.1%), 1.5 ml of thiobarbituric acid (0.8%) and 1.5 ml of 20 % acetic acid (pH 3.5) and then 5 ml of 15:1 v/v butanol-pyridine mixture was added. The absorbance of the organic layer containing the thiobarbituric acid reactive substances (TBARS) was measured at 532 nm[16].

DPPH radical scavenging activity

The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH. About 0.1 mM solution of DPPH in ethanol was prepared and 1 ml of this solution was added to 3 ml of the different concentration of ethanol extract (50-400 µg/ml) in different test tubes. The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using a spectrophotometer. Decrease in absorbance of the reaction mixture indicates higher free radical scavenging activity[17].

Superoxide scavenging activity

This method carried out by using Nitro blue tetrazolium (NBT) reagent, the method is based on generation of superoxide radical (O2-) by auto oxidation of hydroxylamine hydrochloride in presence of NBT, during the reaction the NBT is reduced to nitrite. In brief, aliquots of 0.1 to 1.0 mL to ascorbic acid solution were taken in a test tube, to which 1 mL of sodium carbonate, 0.4 mL of NBT and 0.2 mL of EDTA were added and zero-minute reading was taken at 560 nm. The reaction was initiated by the addition of 0.4 mL of hydroxylamine hydrochloride to the above solution. Reaction mixture was incubated at 25^{0}C for 5 mins; the reduction of NBT was measured at 560 nm. A parallel control was also treated in the similar manner. The ethanol extract was treated in the similar manner, absorbance was recorded and IC_{50} values was calculated[18].

Evaluation of anticancer activity

Procurement of cell lines

In-vitro anticancer activity for ethanol extract was evaluated by MTT assay against MCF-7 breast cancer cell lines. The MCF-7 – human breast adenocarcinoma cell line first isolated in 1970 from the breast tissue of 69 year old Caucasian women were obtained from NCCS, Pune and sub cultured under suitable conditions[19,20].

RESULTS

Preliminary phytochemical investigation

The percentage yield of the EENN was found to be 8.19 % w/w. The preliminary phyto-chemical investigation for the methanol extract of *Nymphaea nauchali* reveals the presence of poly phenols, flavonoids, tannins, steroids, alkaloids and carbohydrates.

Determination of *in-vitro* antioxidant activity

DPPH method

In DPPH assay, the ethanol extract of *Nymphaea nauchali* significantly decreased the absorbance produced by the DPPH and it was found to possess significant IC_{50} value which was comparable to that of standard drug ascorbic acid (See Table No.1).

Lipid Peroxidation

The antioxidant property of ethanol extract of *Nymphaea nauchali* was compared with the standard Ascorbic acid. In lipid peroxidation assay, the EENN and ascorbic acid have significantly inhibited of lipid peroxidation by decreasing the absorbance of the supernatant, The IC_{50} value of EENN was comparable to that of ascorbic acid (See Table No.1)

Superoxide scavenging activity

In superoxide free radical scavenging activity, EENN has offered good antioxidant activity by decreasing the absorbance due to NBT by exhibiting significant IC50 value which was comparable to ascorbic acid (See Table No.1).

Table 1: Effect of methanol extract of *Nymphaea nauchali* on, DPPH, Lipid peroxidation and Superoxide scavenging methods

Sample (µg/ml)	I.C50 (µg/ml)		
	DPPH method	Lipid Peroxidation	Superoxide Scavenging
Ascorbic acid	6.12 ± 0.56	6.95 ± 0.62	35.17±0.23
EENN	25.42 ± 1.81	22.33 ± 1.38	49.66±1.61

All the values are expressed as Mean± SEM, n = 6.

Evaluation of *in-vitro* anticancer activity by MTT assay

The cytotoxic activity of the ethanol extract of *Nymphaea nauchali* on MCF-7 cells was investigated *in vitro* 3-(4) 5-Dimethyl-thiazol-Zyl) - 2,5 biphenyl tetrazolium bromide (MTT).

MTT assay against MCF-7 cells

The significant growth inhibition of breast cancer cell lines was observed due to presence of standard drug Tamoxifen and EENN in a study. The ethanol extract of *Nymphaea nauchali* has shown percentage of inhibition at 500µg/ml was 75.48. Both Tamoxifen and EENN a have exhibited the significant IC_{50} values of 25.43 and 309.09 respectively. (Figure No.1 and Figure No.2)

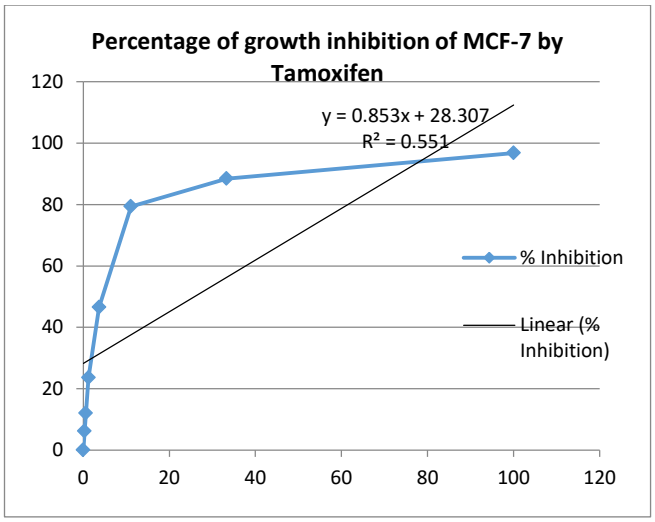

Figure 1: The percentage inhibition of growth of MCF-7 by Tamoxifen

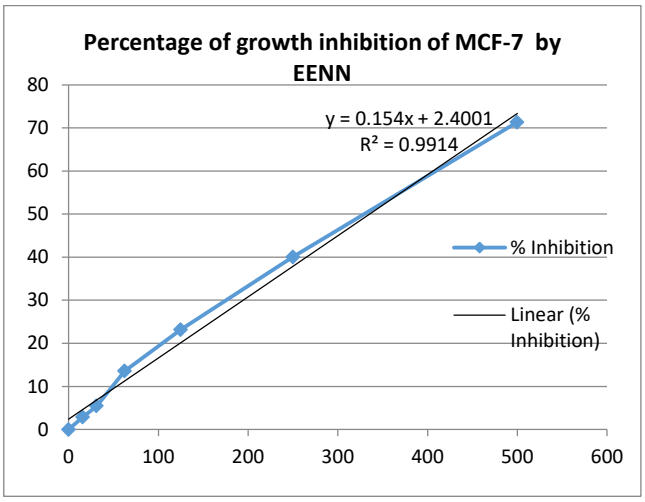

Figure N2: The percentage inhibition of growth of MCF-7 by EENN.

DISCUSSION

Natural products have been regarded as important sources that could produce potential chemotherapeutic agents. Plant derived compounds; in particular, have gained importance in anticancer therapy and some of the new chemotherapeutic agents currently available for use include paclitaxel, vincristine, podophyllotoxin and camptothecin, a natural product precursor from water soluble derivatives[21,22]. Several epidemiological surveys have shown that a diet rich in vegetables and fruits might give protection against tumors by mechanisms that have not been well established yet but probably due to their antioxidant activity. In recent years, naturally occurring plant substances have been getting increased scope for the intervention of malignant invasive progression in the late stage of cancer diseases[23,24,25]. On the basis of this knowledge, certain foods including vegetables, fruits and grains, as well as phyto-constituents of diversified pharmacological properties have been shown to provide a significant protection against various cancers[26,27,28]. Furthermore, there is an increased scope to establish scientific basis on use herbal agents for the management cancers and humans around the globe probably discovered natural remedies against disease and cancer by trial and error over the millennia[29]. Although there are some new approaches to drug discovery, such as combinatorial chemistry and computer based molecular modeling design, none of them can replace the importance of natural products in drug discovery and development. Medicinal plants have long been used to prevent and treat many diseases, including cancer due to their antioxidant potentials and thus they are good candidates for the development of anti-cancer drugs[30,31]. In this regard the study was performed to evaluate the anticancer potentials of the ethanol extract of *Nymphaea nauchali* against human normal, breast, colon, liver and lung cancer cell lines.

MTT [3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] is taken up by the viable cells and reduced to formazan by enzyme succinate-tetrazolium reductase system that belongs to the mitochondrial respiration chain functioning in metabolically active cells. Formazan formed, is a purple coloured water-insoluble product that is largely impermeable to cell membranes and hence resulting in its accumulation within the healthy cells. Hence intensity of purple color and absorbance depends on dead cells. In the present study, we used MCF-7 (breast cancer) cell lines. The ethanol extract was evaluated by MTT assay against all cell lines. The concentration of extract that required to reduce 50% of absorbance (IC_{50}) was recorded against MCF-7 cancer cells[32].

Human breast cancer cells are estrogen receptor (ER)-dependent and carries the wild type tumour suppressor p53 gene[33]. The Tamoxifen is an estrogen receptor antagonist used to treat the breast cancer was used in this study as a reference standard drug. The study revealed that the ethanol extract prepared from the *Nymphaea nauchali* obtained was effective in attenuating the viable tumor cell count in dose dependent manner and shown significant IC50 value which was comparable to results of standard tamoxifen.

According to rule if the crude extract is showing IC_{50} value less than 1mg/mL (1000µg/mL) against MTT assay, then it can be concluding that the plant extract possess significant cytotoxic property[34,35]. In the present study, ethanol extract has shown IC50 values against all the cancer cell lines below 500µg/mL and hence considered as significant values though they are many times more than synthetic standard drug Tamoxifen. In the evaluation of antioxidant activity, ethanol extract of *Tephrosia calophylla* has shown significant antioxidant property by exhibiting significant IC_{50} values against all the three *in vitro* models.

The *Nymphaea nauchali* was essential component of Ayurveda a traditional medicinal system of medicine due to presence of various phytoconstituents for the treatment of various health complications such as diabetes mellitus, ulcers, liver diseases, urinary disorders, cancers and etc. In the present study the ethanol extract was proven for its effective anti-oxidant potentials which is the important mechanism required for the anticancer activity[36,37].

CONCLUSION

The ethanol extract of *Nymphaea nauchali* also shows the significant cytotoxicity against various cancer cells while normal cells were not affected by the extract. But further detailed study is necessary to correlate the anti-oxidant and cytotoxic effects of the extract.

CONFLICT OF INTEREST

All authors are hereby declaring that there is no conflict of interest with respect to manuscript.

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