

Antioxidant and Anticancer Potential of Methanolic Leaf Extract of *Moringa concanensis* Nimmo Against Human Breast Cancer Cell Line MCF-7

S Vijayakumar¹, V Bhuvaneshwari^{1*}, A Sumathi²

¹Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore-641 029, Tamil Nadu, India

²Department of Biotechnology, Hindustan College of Arts and Science, Coimbatore-641 028. TamilNadu, India

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ABSTRACT

Breast cancer is one of the major health problems among women in both developed and developing countries. The objective of the present investigation is focused on the DPPH Radical Scavenging Activity and anticancer effect of the methanolic extract of *Moringa concanensis* Nimmo leaves against Breast cancer (MCF-7) cell line.

The study was facilitated by collecting the plant sample and subjected to crude extraction. The anticancer activity of the crude methanolic leaf extract of *M. concanensis* against MCF- 7 cell line was examined by MTT assay. The present study confirms that the crude leaf extract of *M. concanensis* has potential anticancer activity against MCF- 7 cell lines while compared to the control. *M. concanensis* leaves possess remarkable anticancer property which may lead to development of novel compounds as natural phytomedicine.

Keywords: *Moringa concanensis*, Breast cancer, DPPH, MCF- 7

INTRODUCTION

Cancer is a dreadful diseases characterized by irregular proliferation of the cells¹. Cancer is one of the major health problems of global cancer². Cancer statistics project that by 2030, there will be 26 million new cases and 17 million deaths per year³. Lung, breast and colon cancer are the three most common cancers worldwide, with an increasing annual incidence⁴. In Worldwide, breast cancer is the second leading cause of death in women⁵. Cancer is treated conventionally by immunotherapy, chemotherapy, radiotherapy, surgery and molecular targeting or combination of these methods⁶. About 69% of drugs approved for anticancer medications between 1940 and 2002 are either natural products or developed based on knowledge gained from natural products and phytotherapeutics may contribute much to it⁷.

Antioxidants interfere with the oxidative processes by scavenging free radicals, chelating free catalytic metals and by acting as electron donors⁸. The natural antioxidant mechanisms may be insufficient in variety of conditions and hence dietary intake of antioxidant compounds are important⁹. Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart diseases^{10,11,12,13,14}.

Moringa concanensis belongs to the family Moringaceae which is a plant used in Indian folk medicine for treatment of diseases. Moringa contains 46 bioactive

compounds which help cells to neutralize free radicals. The leaves are highly nutritious, rich in vitamins A, C and E act as a good source of natural antioxidant^{15,16} which included the cure of inflammation, cardiovascular, gastrointestinal, hematological and hepatorenal disorders^{17,18}. Moringa is an important food commodity as all plant parts such as leaf, flower, fruit and immature pods can be used as a highly nutritive culinary vegetable. Almost all *Moringa* species are native to India, from where they have been introduced into several countries of the tropical region^{19,20}.

Leaves of *M. concanensis* help to reduce menstrual pain, blood pressure, constipation, jaundice, skin tumor, diabetes and splenomegaly. Stem bark is used in abortion and fruits are used for joint pains and paralysis and also for curing liver and spleen diseases. Gum is used to treat dental problems. Flowers are used as aphrodisiac, leucorrhoea and abortion²¹ antipyretic, analgesic and anti-inflammatory activity²². Barks and roots are used for the treatment of rheumatism, paralysis, fainting and giddiness, abscess, epilepsy²³. Seed extract of *Moringa* species is reported to have anti-inflammatory properties²⁴, purgative²⁵, tonic¹⁶, analgesic²⁶ and have potential antitumor properties²⁷.

The ethanolic extract of the *Moringa concanensis* was non toxic to normal cell and also have anticancer activities²⁸. Many natural antioxidant and anticancer compounds like succinic acid, Vitamin E, Tetracosanoic acid, trimethylsilyl ester N-Hexadecanoic acid, Phytol, Ethyl 9.cis.,11.trans octadecadienoate, Gamma-sitosterol,

Fucosterol Palmitic acid ester were identified from *Moringa concanensis* through GC- MS analysis²⁹.

Breast cancer is the cancer that develops from breast tissues. Carcinogenesis is composed of a multi-stage process of initiation, promotion and progression. The recognition of tumor development involves an imbalance between cell proliferation and apoptotic cell death, which is the current dogma in tumor biology³⁰.

In the present communication, we report its *in vitro* antioxidant and cytotoxicity activity against different human breast cancer cell line.

MATERIALS AND METHODS

Plant collection and identification

Moringa concanensis Nimmo was collected from Madukkarai hills, Coimbatore District, Tamil Nadu in India, during the session of July to September. The plant was identified and authenticated by Botanical survey of India (BSI), Coimbatore (BSI/SRC/5/23/2015/Tech/861).

Preparation of powder and extraction

Fresh leaves of *M. concanensis* were shade dried and pulverized to powder in a mechanical grinder. About 5g of leaf powder was packed in Soxhlet apparatus and extracted with 300ml of methanol/ethanol. The extract was evaporated using rotary vacuum evaporator. The dried extract was used for *in-vitro* antioxidant and anticancer activity.

DPPH radical scavenging assay

The free radical scavenging activity was tested according to Mensor *et al.*, 2001³¹. Various concentrations of the extracts were mixed with 80 mM of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) in methanol. Then, the solution was incubated for 30 min at room temperature. Ascorbic acid was used as positive control. Absorbance of the solution was measured at 517 nm by a double-beam spectrophotometer. The DPPH radical scavenging activity was calculated using the equation:

Inhibition (%) = $[(A_B - A_A)/A_B] \times 100$, where A_B , absorption of blank sample, A_A , absorption of test sample.

The percentage of DPPH radical scavenging activity was calculated. The 50% inhibitory concentration (IC₅₀) was expressed as the quantity of the extract necessary to react with one half of DPPH radicals.

In-vitro Anticancer activity

Cell line

The human breast adenocarcinoma (MCF7) cell line was obtained from National Centre for Cell Science (NCCS), Pune.

Cell treatment procedure

The MCF cells form monolayer by growing in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS) and maintained at 37°C, 5% CO₂, 95% air and 100% relative humidity. The cells were detached with trypsin-ethylenediaminetetra acetic acid (EDTA) to make single cell suspensions. The counted viable cells were diluted with EMEM containing 5% FBS to give final density of 1x10⁵ cells/mL. In 96-well plate, the cell suspension of 100 µL per well were seeded at plating density of 10,000 cells/well and incubated for 24 h. The cells were treated with serial concentrations of the

test samples in Dimethylsulfoxide (DMSO) and incubated. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT assay

The Cytotoxic activity of crude leaf extract of *M. concanensis* on MCF-7 cells was determined by 3-[4, 5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) assay. After 48 h of incubation, 15µl 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 insoluble purple formazan crystals visualized in light microscope and solubilized in 100µl of DMSO, then measured the absorbance at 570 nm using micro plate reader (Bio-Rad, Hercules, CA, USA). The % cell inhibition was determined by taking vehicle treated control cells (DMSO) were taken as 100% viable using below equation:

% Cell Inhibition = $100 - \text{Abs (sample)/Abs (control)} \times 100$.

Statistical Analysis

Mean, standard deviation was used to calculate the data obtained in the study. IC₅₀ was determined using IC₅₀ Tool Kit³².

RESULT AND DISCUSSION

Antioxidant studies

The results of antioxidant activity using DPPH radical scavenging assay by methanolic and ethanolic extract of *M. concanensis* leaves in comparison with Ascorbic acid was shown in table 1. IC₅₀ value of ascorbic acid, methanolic and ethanolic extract was found to be 239.679 µg/mL, 68.5594 µg/mL and 138.68 µg/mL. The methanolic extract of *M. concanensis* leaves show better antioxidant protection compared to ethanolic extract and the standard.

Table:1 showing result for antioxidant activity using DPPH radical scavenging assay using methanol and ethanol extract.

In-vitro anticancer activity

The results demonstrated that methanolic leaf extract of *Moringa concanensis* had significant cytotoxicity against breast cancer (MCF-7) cell line. Methanolic leaf extracts decreased the viability of MCF-7 cells in a dose-dependent manner. The maximum inhibition of cell growth (47.16±2%) was observed at the concentration of 200 µg/mL of leaf crude extract with IC₅₀ value of 208 µg/mL. The methanolic leaf extracts was decreased the cell viability significantly (p<0.05) in a concentration dependent manner (Table 2). Treatment of *Moringa concanensis* Nimmo leaf extracts (200 µg/ mL) effectively decreased the total number of MCF-7 cells and was accompanied by cell shrinkage, condensed nuclei, blebbing and shape changes when compared to control (Figure 1).

DISCUSSION

Table 1: Radical scavenging Activity.

Concentration of plant extract(μg)	Inhibition % of Ascorbic acid	Inhibition % of Methanolic extract	Inhibition % of Ethanolic extract
50	39.50 \pm 1	49.30 \pm 1	39.50 \pm 1
100	45.10 \pm 2	50.50 \pm 1	45.60 \pm 1
150	48.10 \pm 1	55.60 \pm 1	58.30 \pm 1
200	49.90 \pm 1	65.90 \pm 1	69.70 \pm 1
250	52.60 \pm 1	66.23 \pm 1	69.90 \pm 1

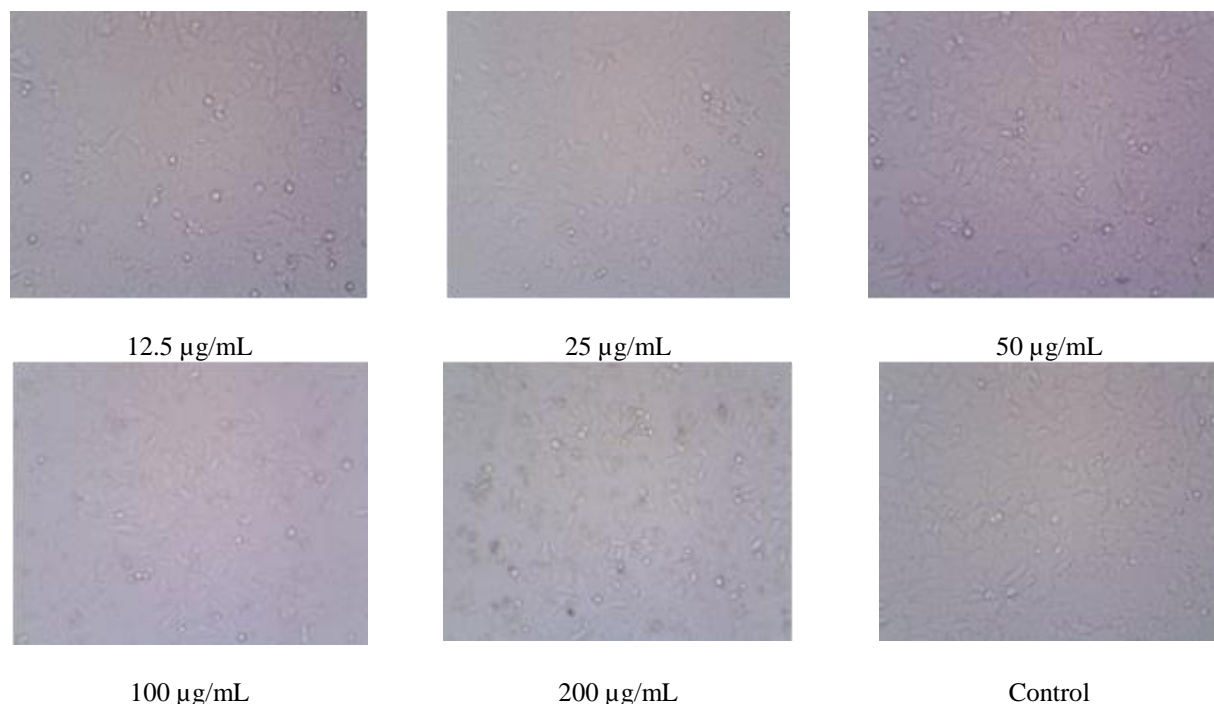
Figure 1: *In-vitro* anticancer activity of *M. concanensis* for MCF-7 cell line.

Table 2: Cytotoxicity of crude methanolic extract against MCF -7 cell line.

Concentration ($\mu\text{g/ml}$)	% Cell Inhibition
12.5	0.3600 \pm 0.001
25	0.2700 \pm 0.01
50	9.8109 \pm 0.1
100	27.1827 \pm 1
200	47.1647 \pm 2
IC ₅₀	208.3

Currently there has been an increased interest globally to identify antioxidant compounds from plant sources which are pharmacologically potent and have low or no side effects for use in protective medicine and the food industry³³. Cytotoxic assays provide important and preliminary information for identifying the plant extract with potential anti-neoplastic properties which can be used for the future studies. In the present report crude methanolic leaf extract of *M. concanensis* shows better antioxidant protection with IC₅₀ of 68.5594 $\mu\text{g/mL}$ and decreases the growth of breast cancer cell line (MCF-7) to about 0.36, 0.27, 9.81, 27.18, 47.16 percentages at the concentration of 12.5, 25, 50, 100 and 200 $\mu\text{g/mL}$ respectively. Charoensin 2014³⁴ reported anticancer and antioxidant activity of methanolic leaf *Moringa oleifera* extract by the radical scavenging activity against 2, 2'-

azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH). It not only exhibit anti-proliferation on cancer cells, but also showed no cytotoxicity on normal cells. Methanolic extract of *Phoenix paludosa* showed a higher cytotoxicity to cancer cell and less cytotoxicity to normal cells³⁵. Similarly, methanolic extract of *Nardostachys jatamansi* act as an accessible source of natural antioxidants and have anticancer activity against breast cancer particularly ER-negative breast carcinom³⁶. The methanolic extract of *Bellis perennis* showed the best anti-proliferative activity against MCF-7 cell line with IC₅₀ value of 71.6 $\mu\text{g/mL}$ ³⁷. Eman et al., 2016³⁸ reported that methanolic extract of *Chrysanthemum coronarium* had potential anti-proliferative activity against several human tumor cell lines. The crude methanolic leaf extracts of *Cocculus hirsutus* demonstrated strong antioxidant and anti-proliferative activities³⁹.

CONCLUSIONS

The present investigation concludes that methanolic extract of *M. concanensis* Nimmo leaf exhibited significant antioxidant activity and inhibition against the breast cancer cell (MCF-7). The results displayed by the crude extracts in the present investigation are promising enough for further isolation and characterization to reveal

any novel metabolite of pharmaceutical importance. Further research is to be carried out to separate and purify the extract, in order to find out the bioactive molecules responsible for the anti proliferation activity observed. The findings from this study indicated that methanol extracts of *M. concanensis* leaf possessed vast potential as a medicinal drug especially in breast cancer treatment.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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