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In-vitro cytoxicity of *M.Maderspantana* (Linn.) Cogn. Fruit Methanol Extract against MCF 7 Human Breast Cancer Cell Line and Quantification of Beta Carotenoid in HPLC

A.Suganthi[1]*, R.Mary Josephine [1]
1.Department of Botany, Nirmala College for Women Coimbatore Tamil Nadu, India

ABSTRACT

The objective of the study was to determine the anticancer efficacy of *M. maderaspatana* fruit extract against Michigan Cancer Foundation-7 (MCF-7). Different concentration (10, 20, 30, 40, and 50 µg/ml) of methanol extract of *M. maderaspatana* fruit were used to assess the *in vitro* cytotoxic effect using 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Identification and quantification of beta carotenoid in HPLC. The various concentrations of crude methanol extract (10, 20, 30, 40, and 50 µg/ml concentration) of *M. maderaspatana* fruit were performed for cytotoxic activity. Effect of inhibition of cell growth showed significantly cytotoxic against Michigan Cancer Foundation-7 (MCF-7) with an inhibit cell growth by 50% (IC50) of 32 ± 1.0 µg/ml. The βeta- carotene content through spectrophotometry and high performance liquid chromatography HPLC analysis showed that 88ppm of betacarotene present in *M.maderspantana* fruits. The results obtained from the study indicate significant cytotoxic activity. The result of anticancer activity study in cell lines of the *M.maderspantana* extract indicates that has anticancer activity against Michigan Cancer Foundation-7 (MCF-7) cancer cell lines. The present study concluded that the methanol extract of *M.maderspantana* possess potent cytotoxic activity.

Keywords: MCF – 7 Breast cancer cell line, *M.maderspantana*, Fruit, Beta carotenoid

*Corresponding Author Email: suganthijrf@gmail.com
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INTRODUCTION

Since ancient times, plants are widely used by humankind for treating various ailments due to its rich chemical potentials and eco-friendly nature. Besides these, herbal molecules also provide leads toward drug development for combating deadly diseases such as cancer and could also find immense use in clinical applications (1). Usage of plants in the management of different diseases and disorders including cancers is the recent approach as observed in the drug development, as it provides a good basis for modern medicines, especially in anti-cancer drug development.

Drug discovery from natural products involves screening for specific and non-specific cytotoxicity against many types of cells. Application of *in vitro* assays for the screening and identification of potential anticancer agents has been a common practice since the beginning of cancer chemotherapy. Chemotherapy is an extremely promising strategy for cancer treatment today, which is defined as the use of synthetic or natural agents (alone or combined) to block the development of cancer in human beings. Today, natural products are focused much for developing cancer chemotherapeutic drugs. Researchers carried on the natural products and drug discovery from seaweeds have resulted in the isolation of over 15,000 novel anti-cancer compounds.

Cancer is a multi-step disease incorporating physical, environmental, metabolic, chemical and genetic factors (2). Breast cancer is the most commonly occurring cancer in women, comprising almost one-third of all malignancies (3). It accounts for approximately 25% of all female malignancies with a higher prevalence in developed countries. Breast cancer is the second leading cause of cancer-related death among females in the world (4). Following genotoxic stress, an intact DNA damage response (DDR) is necessary to eliminate lethal and tumorigenic mutations. Breast cancer is the most frequent malignant tumour in women worldwide (5).

Studies have shown that eating of fruits as a source of food may reduce the risk of heart disease, including heart attack and stroke. Fruits protect the body against certain types of cancers reduce the chances of obesity and type 2 diabetes. It lowers blood pressure, reduces the risk of kidney stones and help to decrease bone loss. Beta carotene is an antioxidant, contributing to protecting the body against the damaging effects of free radicals, which can potentially increase the risk of developing certain diseases, including cardiovascular diseases and cancer.

Melothria maderaspatana (Linn.) Cogn. is a unique place in the Siddha system of medicine. *M. maderaspatana* has been shown to exert hepatoprotective, antioxidant, anti-inflammatory, anti-arthritic activities, immunomodulatory activity (6), antimicrobial activity *(*7) and hypolipidemic (8). Some tribes of India (Orissa) also use this herb for the treatment of diabetes mellitus (9).

Certain traditional medical practitioners also use the leaf-tea of this plant for alleviation of jaundice (10, 11). This plant leaf extract has also anti-arthritic properties (12). Anti-oxidants agents are effective in the prevention and treatment of complex diseases, like atherosclerosis, diabetes, Alzheimer's disease and cancer. Furthermore, our earlier report showed significant antioxidant potential along with higher content of total phenols and flavonoids in *M. maderaspatana* (13).

Even though the rich medicinal properties of *M.maderspantana* fruit have been extensively documented, the anti-cancer potential of this plant has been less explored. Hence, in the present study, further in-depth work is carried out to evaluate the anticancer efficacy of the methanol extract of fruit of *M.maderspantana* and to arrive at the probable mechanism of anti-cancer action of the selected extract.

MATERIALS AND METHOD

Collection and preparation of Plant Material

The *Mukia maderspantana* fruits were collected from Coimbatore district, Tamil Nadu state, India. The fruits were cut into pieces and shade dried. The dried samples were pounded into powder using mortar and pestle. The powder obtained was kept in the laboratory and used for during the period of the research.

Plant Extract

The powdered form (80 g) of stem was extracted using methanol and chloroform (200 ml) for 72 h by soxhlet apparatus. The extract was filtered through Whatman No.1 filter paper and evaporated in a rotarvapour at 40 ºC to get completely dried form. The dried powder was transferred to sterile screw caps and stored at -20°C. At use, the frozen dried extracts were dissolved in media

Determination of Beta carotenoids in HPLC

The chromatographic measurements were performed using Gemini – C18-Reverse phase column - 15cm. The mobile phase consist of Pump A – Acetonitrile; Pump B –Methanol at 50:50 ratios. The separation was performed by gradient elution at a total flow rate of 1 mL/min. The injection volume was 20µl and elutes were detected at 454nm. Betacarotene were as standard for identification and quantification in the *M.maderspantana*. All the standard and sample solutions were filtered through 0.45 membrane filter and injected by auto sample

Cell culture

The Human Breast cancer (MCF-7) cells were procured from the National Center for Cell Sciences (NCCS), Pune, India. The cancer were maintained in Dulbecco's modified eagles medium

(DMEM) supplemented with 2mM l-glutamine and balanced salt solution (BSS) adjusted to contain 1.5 g/L Na_2CO_3, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM l-glutamine, 1.5 g/L glucose, 10 mM (4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid) (HEPES) and 10% fetal bovine serum (GIBCO, USA). Penicillin and streptomycin (100 IU/100µg) were adjusted to 1mL/L. The cells were maintained at 37◦C with 5% CO_2 in a humidified CO_2 incubator.

Evaluation of cytotoxicity

The inhibitory concentration (IC_{50}) value was evaluated using an MTT [3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Cancer cells were grown (1×10^4 cells/well) in a 96-well plate for 48 h in to 75% confluence. The medium was replaced with fresh medium containing serially diluted synthesized compounds, and the cells were further incubated for 48 h. The culture medium was removed, and 100µL of the MTT [3-(4,5-dimethylthiozol-2-yl)-3,5-diphenyl tetrazolium bromide] (Hi-Media) solution was added to each well and incubated at 37 ◦C for 4 h. After removal of the supernatant, 50 µL of DMSO was added to each of the wells and incubated for 10 min to solubilize the formazan crystals. The optical density was measured at 620 nm in an ELISA multiwell plate reader (Thermo Multiskan EX, USA). The OD value was used to calculate the percentage of viability using the following formula.

% of viability = OD value of experimental sample/OD value of experimental control ×100

Morphological study

The MCF-7 cells that were grown on cover slips (1×10^5 cells/cover slip) incubated for 6-24h with compounds at the different concentration, and they were then fixed in an ethanol: acetic acid solution (3:1; v/v). The cover slips were gently mounted on glass slides for the morphometric analysis. Three monolayers per experimental group were photo micro graphed. The morphological changes of the MCF-7 cells were analyzed using Nikon (Japan) bright field inverted light microscopy at 40x magnification.

RESULTS AND DISCUSSION

Betacarotene Contents Of Fruit Extract

The proposed method is a simple, precise and sensitive method for estimation of beta carotenoids from the *M.maderspantana* fruits. The retention time of betacarotene was about 35.65 min. The chromatograms of standard betacarotene and test sample are shown in fig. 3 and 4 respectively. The average betacarotene content in test sample was 88ppm. Beta carotene is an antioxidant, contributing to protecting the body against the damaging effects of free radicals, which can

potentially increase the risk of developing certain diseases, including cardiovascular diseases and cancer The method provides for good resolution and separation of betacarotene .Table 1 and 2 indicates betacarotene peak area and peak height which shows the reliability and suitability of the method.

Cytotoxicity

In vitro cytotoxic activity of *M.maderspantana* methanol extract for the concentrations, 10, 20, 30, 40, 50 µg/ml against MCF – 7 breast cancer cell lines was studied using MTT assay.

(Figs. 1 and 2). Effect of inhibition of cell growth showed significantly cytotoxic against MCF – 7 with an IC50 of 32 µg/ml The result of cytotoxic activity study in cell lines of the *M.maderspantana* fruit extract indicates that has anticancer activity against MCF – 7 breast cancer cell lines.

ANTI-PROLIFERATIVE EFFECTS OF METHANOLIC EXTRACT OF M.MADERSPANTANA FRUIT EXTRACT IN MCF-7 CELLS

Anti-proliferative activity of methanolic extract in *M.maderspantana* MCF-7 cells was determined by MTT assay as shown in (Table 1;Graph 1). Methanolic extract of *M.maderspantana* exhibited a suppressive effect on cell proliferation in dose dependent manner.

Cell proliferation was significantly ($p \leq 0.001$) decreased by viabilities of the rest of the treated groups. 41% at 50 µg of methanolic extract of *M.maderspantana* for 48 h. The IC50 value was determined based on viability rates of cells that treated with varying concentrations of the drug for 48 h. The IC50 was 32 µg for methanolic extract of *M.maderspantana*. The results obtained were in concordance with the anti prolifertive activity of the alcoholic extracts of *Ganoderma lucidum* against the MCF-7 cells where a 70% inhibition was observed at a concentration of 500 µg/mL. (14). According to the type of cancer, different parts of the plant may be efficient for the treatment. Also, it was demonstrated that the fruit of *C.colocynthis* (L.) Schrad can decrease different cancer cell line proliferation (15,16,17)

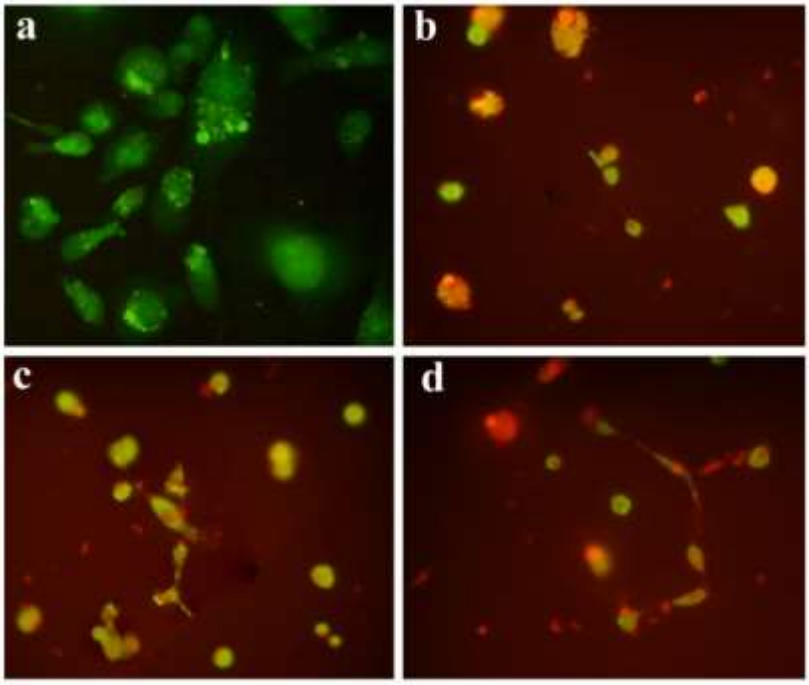


Figure 1: Cytotoxic efficacy of M.maderspantana fruit extract on Michigan Cancer Foundation-7 cell line (a) Normal Michigan Cancer Foundation-7 cell line 2, (b) toxicity at 10µg/ml, (c) toxicity at 20 µg/ml, (d) toxicity at 40 µg/ml

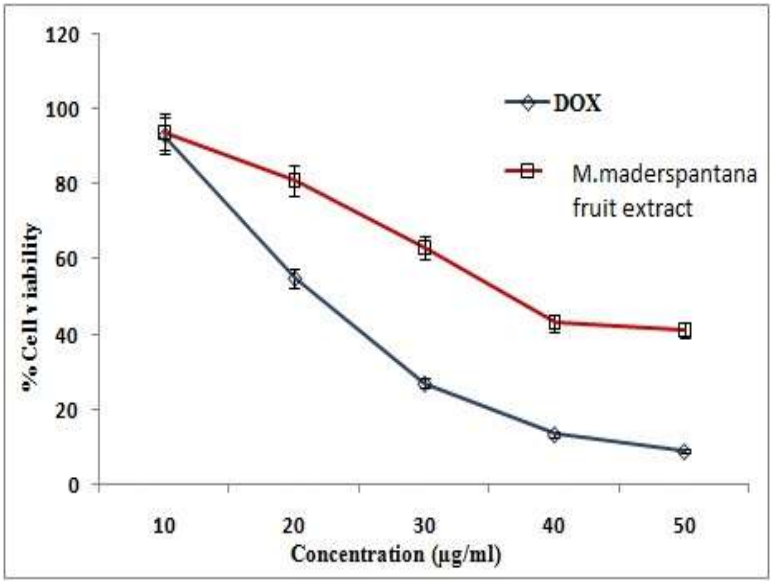
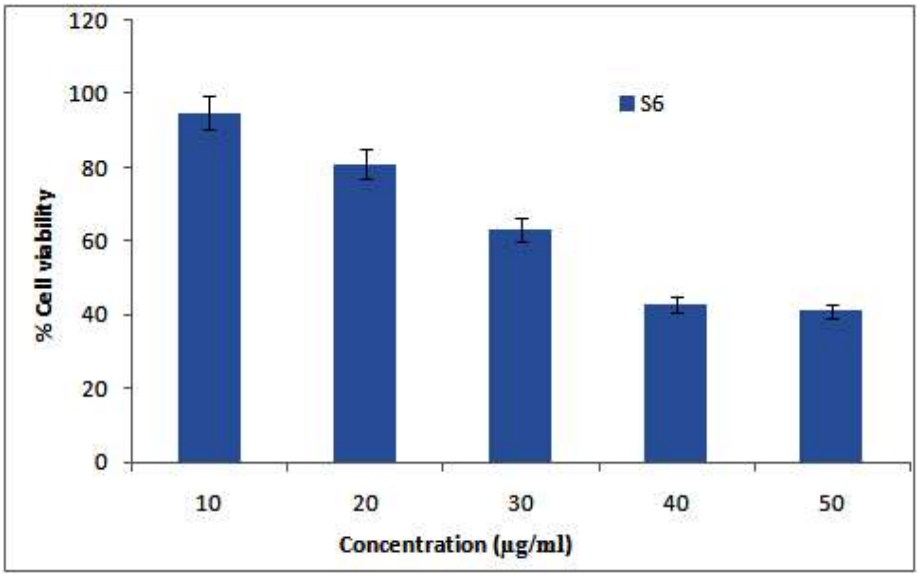


Figure 2: Cytotoxic activity of *M.maderspantana* on MCF -7 cell line



Graph 1: MCF-7 was treated with methanolic extract of *M.maderspantana* .The results represent mean ± SEM for triplicate experiments

Table 1 Percent Cell Inhibition of M.Maderspantana Fruit Extract on MCF – 7 Breast Cancer

Conc (µg/ml)	% Cell Inhibition
10	95
20	81
30	63
40	43
50	41

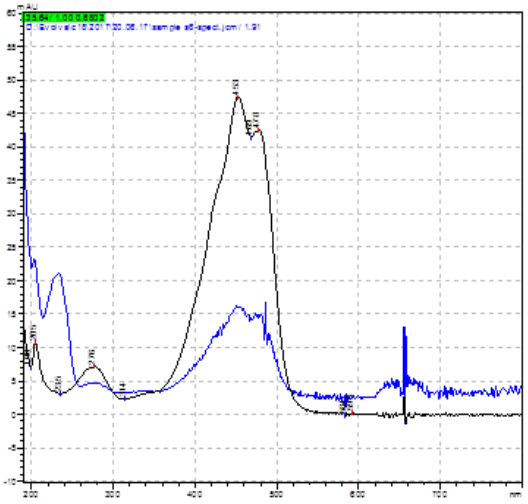


Figure 3

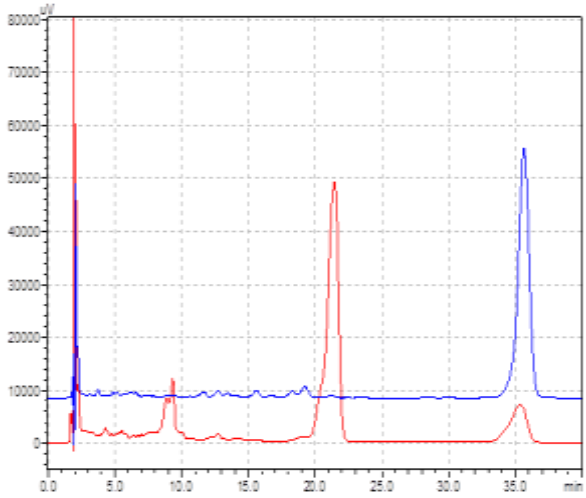


Figure 4

Figure 3:Spectrum of Standard Beta carotene at 454nm *Vs* Spectrum of Beta carotene of *Mukia* maderspantana at 454nm

Figure 4: Standard Beta carotene at 454nm *Vs* Chloroform extract of *Mukia* maderspantana at 454nm

Table 2: Chromatogram of Standard Beta carotene at 454nm

Peak number	Retention time (min)	Peak area	Peak height	Name of the compound
1	35.65	2792658.4	47195.6	Beta carotene

Table 3: Chromatogram of Chloroform extract of *Mukia maderspantana* at 454nm

Peak number	Retention time (min)	Peak area	Peak height	Name of the compound
1	35.36	607933.8	6964.5	Beta carotene

Calculation

20µL of sample contains 3425 µg of raw material concentration.

$$\beta \text{ carotene percentage} = \frac{\text{Area of sample}}{\text{Area of standard}} \times \frac{\text{Concentration of standard}}{\text{Concentration of sample}} \times \text{Purity of standard}$$

$$= \frac{607933.8}{2792658.4} \times \frac{1.43}{3425} \times 97\%$$

= 0.0088%

= 88 ppm

CONCLUSION

In the present studies, *Melothria maderaspatana* extract was selected and screened for its β-carotene and cytotoxic effect against MCF7 cell lines with a view to suggest an anti- cancer herbal drug for managing breast cancer.

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