

VOLUME 8 ISSUE 2 2022

ISSN 2454-3055



**INTERNATIONAL
JOURNAL OF
ZOOLOGICAL
INVESTIGATIONS**

***Forum for Biological and
Environmental Sciences***

Published by Saran Publications, India



International Journal of Zoological Investigations

Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India



ISSN: 2454-3055

Antioxidant and Antiproliferative Activity of Noni Fruit (*Morinda citrifolia*) Extracts Against Human Breast Cancer Cell Line (MCF-7)

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Received: 4th June, 2022; Accepted: 11th July, 2022; Published online: 21st July, 2022

<https://doi.org/10.33745/ijzi.2022.v08i02.011>

Abstract: Noni juice, obtained from the fruit of the noni tree (*Morinda citrifolia* L.), is a popular commodity in the market, particularly in the South Pacific. It is widely used by consumers for the prevention of several lifestyle diseases. Although there is increasing interest in the potential therapeutic use of noni plants, there are no comparative studies on the various commercialized noni fruit juices available to decipher their phytochemical composition and properties against carcinomas. The present study, therefore, aimed to investigate the juice's anecdotal use as complementary alternative medicine to manage cancer. In this study, we evaluated the cytotoxicity and antioxidant activity of *M. citrifolia* fruit extracts. DPPH (2,2-Diphenyl-1-picrylhydrazyl) tests were used to assess the antioxidant of the extracts. The cytotoxicity of the MCF-7 breast cancer extracts was performed using the MTT procedure. Antioxidant and anticancer studies showed the potential activity of *M. citrifolia* fruit extracts. Further work involving more extensive *in vitro* and *in vivo* studies are needed to elucidate its anticarcinogenic activities.

Keywords: *Morinda citrifolia*, Breast cancer, Noni juice, Antioxidant, 2,2-Diphenyl-1-picrylhydrazyl

Citation: Rengaswamy Gopal, Remya Vijayakumar, Karkuzhali M., Sharmila J. and Ashok K.: Antioxidant and antiproliferative activity of noni fruit (*Morinda citrifolia*) extracts against human breast cancer cell line (MCF-7). Intern. J. Zool. Invest. 8(2): 73-84, 2022.

<https://doi.org/10.33745/ijzi.2022.v08i02.011>



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Introduction

Natural products from plants have played major, sustaining roles in the lives of humans, especially for food sources and for medicinal products. Natural products have, until recently, been the primary source of commercial medicines and drug

leads. A recent survey revealed that 61% of the 877 drugs introduced worldwide can be traced to or were inspired by natural products (Sina *et al.*, 2021). However, beginning in the 1990s, natural product drug discovery was virtually eliminated in

biggest pharmaceutical companies. This was primarily due to the promise of the then-emerging field of combinatorial chemistry, whereby huge libraries of man-made small molecules could be rapidly synthesized and evaluated as drug candidates (Krishnaiah *et al.*, 2012).

Thus far, this approach has led to lukewarm results at best. From 1981 to 2002, no combinatorial compounds became approved drugs, although several are currently in late-stage clinical trials. At the same time, the number of new drugs entering the market has dropped by half, a figure of which the large pharmaceutical corporations are painfully aware. The haystack is larger, but the needle within it is more elusive. This has led only recently to a newfound respect for the privileged structures inherent within natural products (Zhu *et al.*, 2020).

Of the roughly 350,000 species of plants believed to exist, one-third of those have yet to be discovered. Of the quarter million that have been reported, only a fraction of them have been chemically investigated. Food plants and culinary herbs and spices are known to contain myriad Phytochemicals with medicinal properties (Zhu *et al.*, 2020).

Morinda citrifolia Linn an Indian mulberry or Noni in English and Mancanaari in Tamil is the plant which is used as a raw material for nutraceutical and functional food products. There are more than 120 nutraceutical compounds identified in Noni (Agostini-Costa *et al.*, 2012). Noni was the most popular plant used as food, drink, medicine and dye (Ahmad *et al.*, 2011). Noni is native from Southeast Asia to Australia and is cultivated in Polynesia, India, the Caribbean, Central and northern South America (Akihisa *et al.*, 2007). Various parts of the plant, including its leaves, fruit, bark and roots, have been used for over 2000 years to treat several diseases such as high blood pressure and diabetes, and to cure eye problems, skin wounds, throat problems, respiratory ailments, constipation, and stomach pains (Assi *et al.*, 2017). Noni juice has been accepted in the European Union as a novel food

(Basar *et al.*, 2010). In the United States alone, 19 patents have been registered by the US Patent and Trademark Office since 1976 (Beh *et al.*, 2012).

Noni or Yor juice extract which is obtained from fermented Noni fruits is the most effective product that has helped relieved people (n ≥ 10,000) from the suffering of about 22 conditions, such as arthritis, heart disease, diabetes, headache and muscle pain, high blood pressure, cancer etc. (Brown, 2012).

Morinda citrifolia (Noni) has been utilized for a considerable length of time to cure or counteract assortment of diseases by conventional therapeutic professionals in Hawaii and Polynesia. A review on *Morinda citrifolia* has been published by Assi *et al.* (2016) which described the antimicrobial and antiseptic activity, antifungal activity, antioxidant activity, anti-inflammatory activity, anti-arthritic activity, anti-cancer activity, antidiabetic activity, wound healing activity, memory enhancing activity, anxiolytic and sedative activity, analgesic activity, gastric ulcer healing activity, antiemetic activity, gout and hyperuricemia healing activity, immunity enhancing activity, anti-viral activity, anti-parasitic activity, anti-tuberculosis activity, osteoporotic and otoscopic enhancer. These activities were carried out to the extent of *in vitro*, *in vivo* and clinical trial stages (Chan-Blanco *et al.*, 2006).

Antioxidants may serve the task of reducing oxidative damage in humans induced by free radicals and reactive oxygen species under oxidative stress conditions. These conditions can cause DNA and protein damage, lipid peroxidation, cancer, ageing and inflammatory activity (Kalandakanond *et al.*, 2004). Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (Hirazumi and Furusawa, 1999). An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 2,2-diphenylpicrylhydrazyl (DPPH) stable radical spectrophotometrically. In the presence of an antioxidant, DPPH radical

obtains one more electron and the absorbance decreased (Kalandakanond-Thongsong and Charoenphandhu, 2012)

Antioxidants are also commonly utilised to preserve health and prevent illnesses such as cancer or other coronary heart problems as components in dietary supplements (Muralidharan and Srikanth, 2010). The cell damaging effects of free radicals are stopped or reduced by antioxidants. Antiphenols, flavonos, saponins, glycosides, and tannins in the plant extracts can be the source of antioxidants. Toxicity and safety of herbal medicines are the main issue in the western countries. Several studies have shown that plants generate powerful, thus essential sources of antioxidants (Narasingham *et al.*, 2016).

Some scientists have recently indicated that it is not possible to quantify antioxidant activity precisely and in quantitative terms using a simple universal approach (Palu, 2009). It is also recognised that plants that have potential antioxidant action also have anti-cancer activity. Many ROS cause oxidative damage to the human organism. Some of the most relevant free radicals in their physiological field are hydroxylate, Nitric Oxide, Superoxide Anion, Peroxylate and Nitrogen Dioxide, and Non-radicals like, Hydrogen peroxide, Hydrochloric Acid, singlet oxygen, ozone (Pawlus and Kinghorn, 2007). Superoxide anion radical, the oxygen one electron reduction product, is the leading source of radicals that are purposefully or mistakenly generated by the decrease in molecular oxygen through various physiological processes (Pawlus *et al.*, 2005).

Cancer is the leading cause of death (18.4% of all deaths; colorectal 9.2% and stomach cancers 8.2%) (WHO, 2020). In spite of good advancements for diagnosis and treatment, cancer is still a big threat to our society. This is the second most common disease after cardiovascular disorders for maximum deaths in the world. It accounts for about 23% and 7% deaths in USA and India, respectively. The world's population was expected to be 7.5 billion by 2020 and

approximations predicted that about 15.0 million new cancer cases will be diagnosed; with deaths of about 12.0 million cancer patients (Sreenivasulu, 2015).

Though progress in reducing lung cancer deaths has improved due to declines in smoking and advances in early detection and treatment, however, progress in reducing colorectal, breast, and prostate cancers has slowed (Srikanth and Muralidharan, 2009). There are so many methods for the treatment of cancer like they involve surgery of tumor, radiotherapy, immunotherapy, chemotherapy, cancer vaccinations, photodynamic therapy, stem cell transformation or combination thereof often accompanied by severe side effects. Such side effects include limited bioavailability, toxicity, no specificity, fast clearance and restriction in metastasis. Treatment methods depend upon the cancer type, stage and location. Chemotherapeutic agents involve cytostatic and cytotoxic drugs which have shown promising results alone or in combination with other cancer therapies. These chemotherapeutic agents involve topoisomerase inhibitors e.g. irinotecan (side effects include: neutropenia, sensory neuropathy, and diarrhoea) and doxorubicin (side effects include cardiotoxicity), alkylating agents e.g. oxaliplatin, melphalan, carboplatin, cisplatin and cyclophosphamide (side effects include: nephrotoxicity, gastrointestinal toxicity, cardiovascular toxicity, pulmonary and hematologic toxicity), microtubules acting agent e.g. vincristine, vinblastine, docetaxel and paclitaxel etc. (Wang *et al.*, 2000).

Materials and Methods

MATERIALS AND METHODS

Collection and identification of plant material:

The fruit of *Morinda citrifolia* was collected from Puzhal lake, Chennai, Tamil Nadu, India and were authentically identified by Prof. P. Jayaraman, Institute of Herbal Botany, Plant Anatomy Research Centre, West Tambaram, Chennai, Tamil Nadu, India.

Preparation of plant extract:

Extraction of *Morinda citrifolia* fruit using different solvent was done according to the method of Medhe *et al.* (2014). The aqueous, chloroform and methanol extracts of fruits were prepared by dissolving 100 g of fine powdered fruit material. The contents were kept in orbiter shaker for 48 h. Then the extracts were filtered and it is dried in hot air oven at 37 °C. Then the extract was stored under refrigeration at 4 °C for further studies.

Phytochemical profiling:

Phytochemical screening of *Morinda citrifolia* extracts (aqueous, chloroform, and methanol) was carried out as described by Calani *et al.* (2013).

In vitro Antioxidant potential of the whole plant extracts:

DPPH Assay:

The antioxidant potential of the extracts was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity by the modified method of McCune and Johns (2002).

The reaction mixture (3.0 ml) consisting 1.0 ml DPPH in methanol (0.1 mM), 1.0 ml methanol and 1.0 ml different concentrations of the extracts (20, 40, 60, 80 and 100 µg/ml) was incubated in dark for 10 min., and the optical density (OD) was measured at 517 nm against blank. For control, 1.0 ml of methanol was used in place of isolated compounds. L- Ascorbic acid was used as positive control. Percentage inhibition of DPPH was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{\text{OD of Control} - \text{OD of Experiment}}{\text{OD of Control}} \times 100$$

Oral cancer (MCF-7) cell line was purchased from NCCS, Pune. Dulbecco's modified Eagle's medium (DMEM), trypsin, ethylenediamine tetraacetic acid, sodium bicarbonate, propidium iodide (PI), RNase, triton X-100, thiazolyl tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), penicillin and streptomycin were purchased from Sigma Aldrich. Fetal bovine serum (FBS), Agarose, Propidium iodide (PI), buffered saline (10X) was purchased

from Gibco, Invitrogen Life Technologies and solvents like methanol, chloroform, hexane and ethyl acetate were purchased from Hi Media.

Culture of MCF-7 cells:

MCF-7 cells were grown in DMEM containing 10% FBS, 10,000 IU/ml penicillin and 10,000 µg/ml streptomycin in a 25 cm²-culture flask in a CO₂ incubator at 37 °C and 5% CO₂ under controlled humidified atmosphere. Once the cells reached ~90% confluency, they were trypsinized using trypsin (0.05%) – EDTA (0.54 mM) solution, washed thoroughly with media and subcultured into a 75-cm² culture flask for expansion. This process was repeated twice till the cells attained a consistent growth phase. Once after the cells attained consistent growth phase, they were trypsinized at 80% confluence and then utilized for the various assays.

Assessment of cytotoxicity by MTT assay:

The MCF-7 cells were trypsinized when they were at 80% confluence and seeded in a 96-well plate at the density of 7 x 10³ cells/well. The cells were incubated in a CO₂ incubator at 37 °C and 5% CO₂ under controlled humidified atmosphere overnight for attachment. After overnight incubation, the cells were exposed to the solvent extract at different concentrations (25, 50, 75, 100 and 125 µg/ml, respectively for 24 h. 50 µl MTT (5 mg/ml stock) was added to the cells and further incubated for 3 h at 37 °C. At the end of incubation period, the contents of the plate were discarded by simple decantation and the plates were dried overnight at room temperature. The purple-coloured formazon crystals formed were dissolved in 100 µl of DMSO by shaking at 400 rpm for 15 min at RT in a thermo shaker. The intensity of the colour developed was absorbed at 570 nm in a multimode microplate reader. Percentage of cell viability was calculated as follows:

$$\text{Cell viability (\%)} = \frac{\text{OD of Experiment}}{\text{OD of Control}} \times 100$$

The percentage growth, percentage inhibition and 50% growth inhibition (IC₅₀) values were calculated using a pre-programmed MS-Excel template.

Cytomorphological (CT) Studies:

The CT changes of extract treated MCF-7 cell line were assessed. Cancer cells (1×10^6 cells/ml) were plated in 100 mm dishes and incubated for 24 h under controlled environment. Then, the spent medium was removed, followed by addition of fresh medium with or without extracts at 25 h 50% inhibitory concentration and incubated for 24 h. After incubation, the cells were visualized under inverted light microscope at 10 X magnification.

Assessment of cell viability by PI staining:

PI staining of MCF-7 cell was done according to the method of Spector *et al.* (2001).

Statistical Analysis:

The data of DPPH and MTT assays were subjected to statistical analysis and the Mean and SE for five individual observations was calculated. The significance of the sample mean was tested by Two Way ANOVA using SPSS software. The differences were considered as significant at $p < 0.05$ level.

Results

Yield of Fruit Extracts:

The yield of *Morinda citrifolia* extracts was maximum in methanol (2.5%), followed by chloroform (1.5%), and aqueous (1.5%). The colour of extracts was dark brown and the consistency was paste (Table 1).

Qualitative phytochemical profiling:

Phytochemical screening for the solvents used in the present (Aqueous, chloroform, and Methanol) showed the presence of Carbohydrates, tannins, saponins, flavonoids, anthocyanin, glycosides, cardiac glycosides, terpenoids, triterpenoids and phenols. Whereas methanolic extract showed the presence of all the secondary metabolites. Methanol extracts exhibited only 16 highly positive preliminary phytochemical tests. Collectively (Table 2) Methanol extract showed more positive results when compared to other extract such as aqueous and chloroform.

Antioxidant assay:

Antioxidant Potential of M. citrifolia fruit extracts:

The data on per cent inhibition of DPPH by Aqueous, chloroform and methanol extract along with L-Ascorbic acid (standard) is presented in Table 3 and Figure 1. The results depicted a dose-dependent inhibition in DPPH activity in all the extracts tested; the inhibition being higher in methanol extract than that of aqueous and chloroform extracts. The IC_{50} value of DPPH inhibition was observed at 68.085 $\mu\text{g/ml}$ concentration of methanol extract and 80.366 $\mu\text{g/ml}$ concentration of aqueous extract and 97.209 $\mu\text{g/ml}$ concentration of chloroform extract. In the case of standard Ascorbic acid, the IC_{50} value was 67.649 $\mu\text{g/ml}$. When compared among all the three extracts, methanol extract of *M. citrifolia* alone showed highest inhibition than the other extracts. Analysis of two-way ANOVA revealed that the results are significant at $P < 0.05$. The changes in DPPH activity are significant within various concentrations.

The results showed that the methanol extract of *M. citrifolia* has better antioxidant potential with respect to L- Ascorbic acid (standard). The calculated IC_{50} value of methanol extract was 68.085 $\mu\text{g/ml}$ and for standard it was 67.649 $\mu\text{g/ml}$. The value reasonably showed that the methanol extract of *M. citrifolia* extract has more antioxidant potential and hence in future it may be used as a potent antioxidant drug.

Cytotoxicity assay (MTT Assay):

The 24 h IC_{50} value of MCF-7 cells was 74.276 $\mu\text{g/ml}$ for methanol extract, 86.848 $\mu\text{g/ml}$ of aqueous extract and 91.317 $\mu\text{g/ml}$ of chloroform. From these results it was obviously clear that the methanol extract of *M. citrifolia* has profound effect in controlling MCF- 7 cell proliferation. Likewise, the 24 h treated aqueous extract also showed the better activity when compared to chloroform extract. The data altogether depicted that the methanol extract and the other extracts tested also significantly controls the cell proliferation of MCF-7 cells even at very lower

Table 1: Yield of solvent extracts of *M. citrifolia*

S. No.	Solvents	Weight of dried extract (g)	Yield (%)	Colour	Consistency
1	Aqueous	100	1.5	Dark brown	Paste
2	Chloroform	100	1.5	Dark brown	Paste
3	Methanol	100	2.5	Dark brown	Paste

Table 2: Qualitative phytochemical analysis of *M. citrifolia* fruit extracts

S. No.	Secondary metabolites	Aqueous	Chloroform	Methanol
1	Carbohydrate	+++	+++	+++
2	Tannins	+	+	+
3	Saponins	++	+	++
4	Flavonoids	+++	+	+++
5	Alkaloids	+	-	+++
6	Anthocyanin	++	-	+++
7	Quinones	-		+
8	Glycosides	+	+	+++
9	Cardiac glycosides	+	+	++
10	Terpenoids	++	+	++
11	Triterpenoids	+	+	++
12	Phenols	+	+	++
13	Coumarins	-	-	++
14	Acids	-	+	+
15	Protein	-	-	+
16	Steroids	-	-	+++

+++ strongly present; ++ positive; + present; - absent

Table 3: Antioxidant Potential of *M. citrifolia* fruit extracts

Concentration	Aqueous extract	Chloroform	Methanol	Ascorbic acid
20 µg/ml	0.933±0.001* (-12.280)	0.944±0.001* (-11.278)	0.810±0.0005* (-23.809)	0.869±0.002* (-18.264)
40 µg/ml	0.816±0.0006* (-23.276)	0.867±0.004* (-18.483)	0.705±0.0007* (-33.740)	0.772±0.001* (-27.381)
60 µg/ml	0.702±0.0007* (-34.022)	0.730±0.002* (-31.328)	0.589±0.003* (-44.642)	0.604±0.001* (-43.170)
80 µg/ml	0.536±0.002* (-49.624)	0.643±0.001* (-39.567)	0.448±0.001* (-57.894)	0.414±0.002* (-61.027)
100 µg/ml	0.318±0.001* (-70.112)	0.514±0.002* (-51.691)	0.205±0.002* (-80.670)	0.171±0.019* (-83.928)
IC ₅₀ µg/ml	80.366	97.209	68.085	67.649

Values are mean + S.D. of five observations; Values in parentheses are per cent change over control; - Denotes per cent decrease over control; * indicates significance at P<0.05.

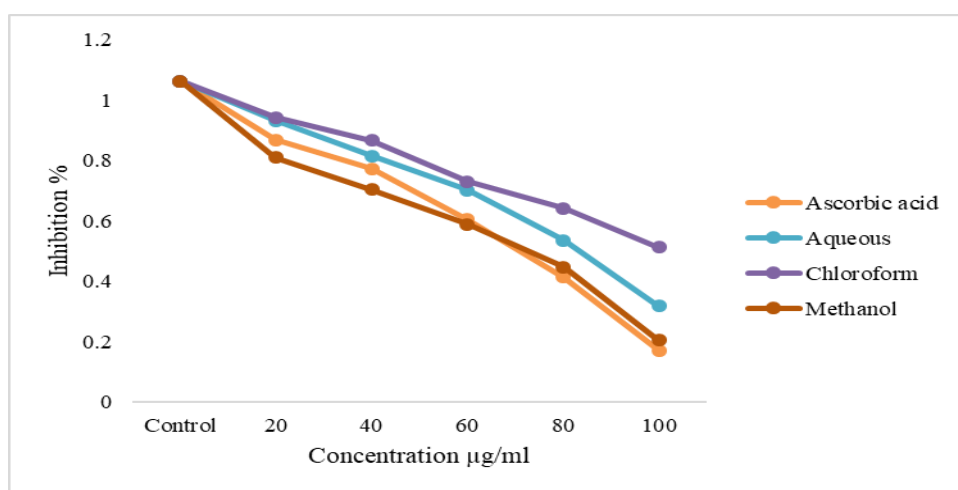


Fig. 1: Antioxidant potential of *Morinda citrifolia* fruit extracts.

concentration. Hence in future, the *M. citrifolia* plant extracts can be used as a potent anticancer agent Table 4 and Figures 2-5.

Nuclear Staining and Apoptotic Morphology Studies:

Since we got good results in 24 h IC₅₀ concentrations of 74.276 µg/ml for methanol extract of *M. citrifolia*, further studies were done only in the above-mentioned concentrations. Apoptotic nuclear staining by using PI stain revealed nuclear morphological changes, which were observed under fluorescent microscope. Results for the control and experimental groups

on the percentage of MCF- 7 cells displaying apoptotic nuclear morphology are depicted in Figure 6.

A progressive increase in the number of positive cells were observed in methanol extract treated cells. The control cells had intact nucleus, whereas the treated cells showed altered cell morphology with intense nuclear pyknosis as signs of apoptosis by PI staining.

Discussion

Essential bioactive phytochemicals confirmed in the present study include alkaloids, carbohydrates

Table 4: Anticancer activity of *M. citrifolia* fruit extracts against MCF-7 cells

Concentration	Aqueous extract	Chloroform	Methanol
20 µg/ml	95.034±0.009* (-4.966)	93.3±0.240* (-6.7)	92.612±0.132* (-7.388)
40 µg/ml	84.104±0.006* (-15.896)	86.73±0.330* (-13.27)	80.578±0.218* (-19.422)
60 µg/ml	72.146±0.002* (-27.854)	71.738±0.396* (-28.262)	64.878±0.282* (-35.122)
80 µg/ml	57.168±0.022* (-42.832)	59.112±0.062* (-40.888)	44.036±0.448* (-55.964)
100 µg/ml	36.236±0.016* (-63.764)	43.01±0.250* (-56.99)	20.448±0.382* (-79.552)
IC ₅₀	86.848	91.317	74.276

Values are mean + S.D. of five individual observations; Values in parentheses are per cent change over control; - Denotes per cent decrease over control; * indicates significance at P<0.05.

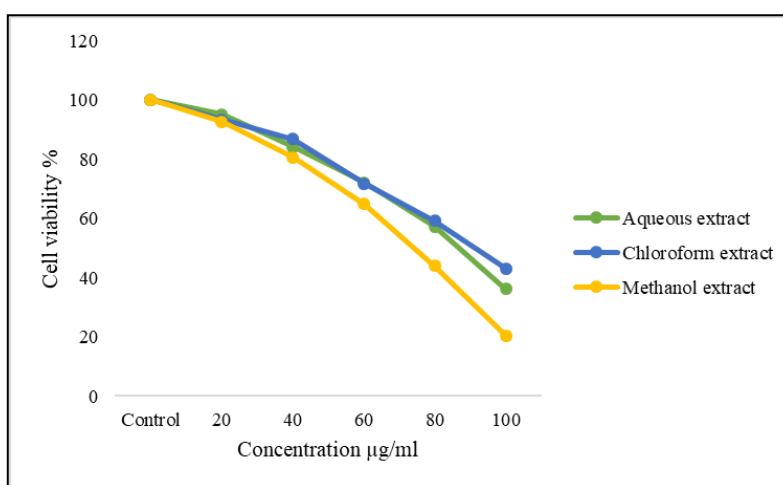


Fig. 2: Anticancer activity of *M. citrifolia* fruit extracts against MCF-7 cells.

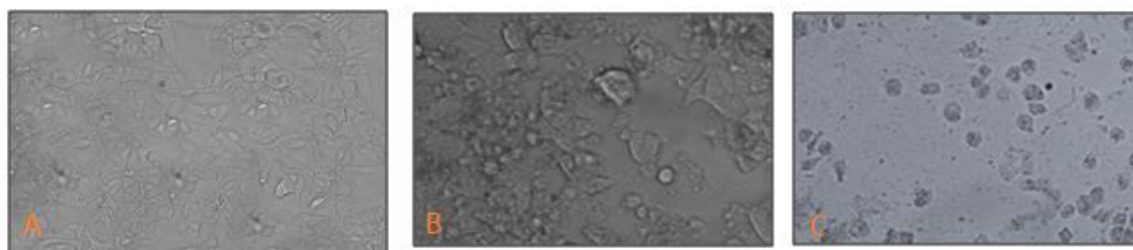


Fig. 3: Anticancer activity of *M. citrifolia* aqueous extract against MCF-7 cells. A- Control MCF- 7 cells; B- 86.848 µg/ml; C- 100 µg/ml.

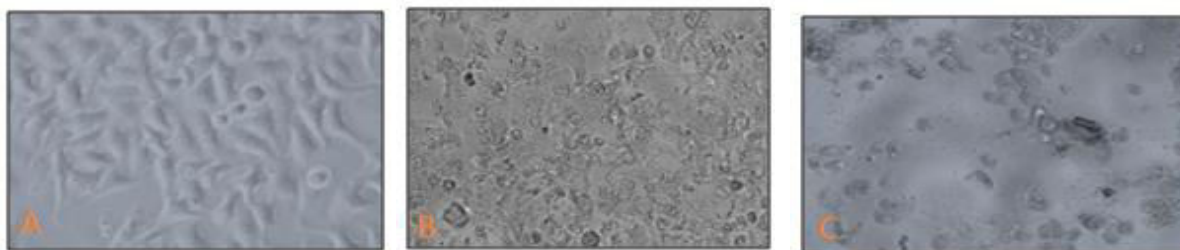


Fig. 4: Anticancer activity of *M. citrifolia* chloroform extracts against MCF-7 cells. A- Control MCF- 7 cells; B- 91.317 µg/ml; C- 100 µg/ml.

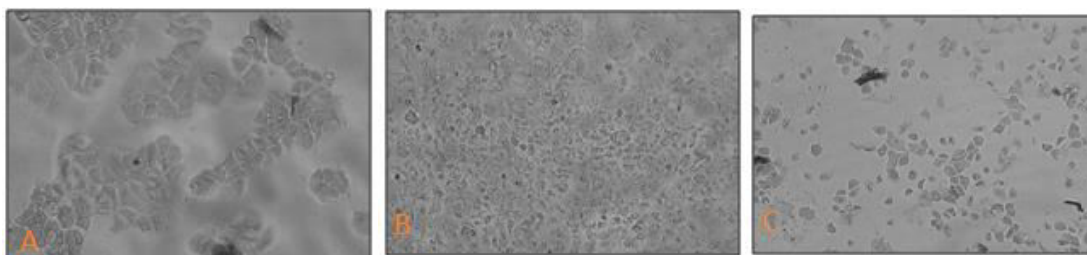


Fig. 5: Anticancer activity of *M. citrifolia* methanol extracts against MCF-7 cells. A- Control MCF-7 cells; B- 74.276 µg/ml; C- 100 µg/ml.

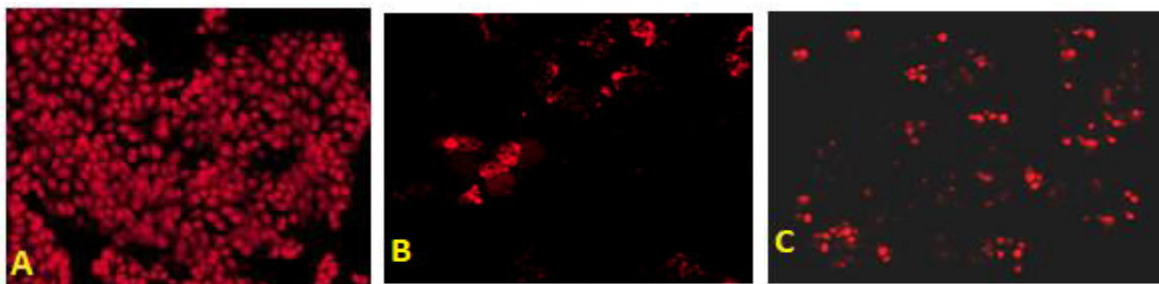


Fig. 6: Anticancer activity of *M. citrifolia* methanol extracts against MCF-7 cells. A- Control MCF-7 cells; B- 74.276 µg/ml; C- 100 µg/ml.

steroids, tannins, saponins, flavonoids, anthocyanin, quinones, glycosides, glycosides, terpenoids, phenols, coumarins and acids. These compounds are a well-established bioactive compound with a wide variety of medicinal applications. The phytochemical content in the present study was compared with the available literature for phytochemical experiments of *M. citrifolia* root extracts. and excavations revealed the presence of active phytochemicals. Chemical elements at the roots of *M. citrifolia* was also reported by William *et al.* (2012).

The polyphenolic compounds known for their chemical properties have been identified in different solvent components of this plant. Terpenoids have been found in high concentrations of many soluble solvents and are known to have a wide range of biological functions including antimicrobial, antifungal, anti-parasitic, antiviral, antiallergenic, antispasmodic, anti-hyperglycemic, anti-inflammatory, immunomodulatory and antibacterial and antibacterial, antimalarial properties against cancer (Samoylenko *et al.*, 2006). Based on the presence

of many bioactive compounds further research on the antioxidant potential was performed. Medicinal plants are among the best sources of these antioxidant compounds for their availability and easy access (Siddiqui *et al.*, 2007). Researchers have turned to herbal remedies because the use of synthetic antioxidants poses a serious threat such as carcinogenicity. Therefore, it is important to perform research on medicinal plants that are recognized for their antioxidant capacity, in addition to their normal medicinal functions. Therefore, they can be useful in the treatment and management of complex diseases such as autoimmune disease and cancer (Singh, 2012).

Methanol extract of *M. citrifolia* was also investigated to determine its mechanism of cell death by looking at it under a distorted microscope. From very small images it was observed that MCF-7 cells lose their normal shape when exposed to IC₅₀ and high concentration of methanol extraction 24 h. These morphological changes in MCF-7 cells were consistent with previous studies suggesting that this type of

mutation could be a mechanism of cell death (Takashima *et al.*, 2007).

Polyphenolic compounds may inhibit cancer cells by xenobiotic metabolizing enzymes that alter the metabolic activity of potential carcinogens, while other flavonoids may also alter hormone production and inhibit aromatase inhibition. The mechanism of action of phenol cancer-fighting activity may be to disrupt cell division during mitosis in the telophase phase. It has also been reported that phenols reduce the amount of cellular protein and mitotic index and colon formation during the proliferation of cancer cells (Su *et al.*, 2005).

The *in vitro* cell proliferation assay supported their significant cytotoxic environment against the MCF7 cell line. Methanol treated cell, which shows better performance with lower IC₅₀ values compared to high concentrations. Morphological analyses using a reverse-phase microscope, processes to PI contamination with a fluorescence microscope, showed that the release of methanol was able to cause the death of MCF-7 breast cancer cells through apoptosis in a dose-dependent manner. In conclusion, our results showed that methanol release significantly reduced cell function, and altered the cellular morphology of MCF-7 cells in a concentrated manner. The data showed that exposure to methanol extraction was most effective in MCF-7 cells. More molecular research is needed to determine the mechanism of action of methanol extracted from human breast cancer cells. Although the findings confirm claims that selected herbal products are cytotoxic to cancer cells, *in vivo* studies should be performed to confirm the potency and toxicity of these herbal products. It is wise to do *in vivo* studies (Srikanth and Muralidharan, 2009).

Conclusion

In conclusion, natural antioxidants have many important applications in health promotion, food preservation, food flavouring and cosmetics. They are preferred over synthetic

antioxidants because they are safer for consumption and more environmentally friendly. The present study investigates the antioxidant activity and polyphenolic content of medicinal plants from India. We found extracts rich in antioxidants and in polyphenols, which merit further investigations. The extracts *M. citrifolia* showed the greatest antioxidant, and antiproliferative activities, a discovery that makes this species a promising source of anticancer agent development especially for colon and liver cancers, and hence worthy of further investigation. Isolation of active compounds and exploring their mode of action against tumours by using *in vivo* experimental models would make an important future study.

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