Anticancer Activity of Noni Fruit (*Morinda citrifolia*) Extracts Against Human Hepatocellular Carcinoma Cell Line (HepG-2) and its Apoptotic Mechanism

Rengaswamy Gopal1*, Remya Vijayakumar2, Sharmila J.1, Karkuzhali M.1 and Ashok K.3

1Department of Zoology, Quid-E-Millath Government College for Women (A), Chennai, Tamil Nadu, India
2Department of Zoology, Annamalai University, Annamalai Nagar, Tamil Nadu, India
3Department of Microbiology and Biotechnology, Faculty of Arts and Science, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India

*Corresponding Author

Received: 12th June, 2022; Accepted: 16th July, 2022; Published online: 26th July, 2022

https://doi.org/10.33745/ijzi.2022.v08i02.014

Abstract: *Morinda citrifolia* L. (Noni) fruits have been used for thousands of years for the treatment of many health problems including cancer, cold, diabetes, flu, hypertension, and pain. Plant extracts have reported for several therapeutic benefits, but extraction of individual compound from the extract often exhibits limited clinical utility as the synergistic effect of various natural ingredients gets lost. They generally constitute polyphenols and flavonoids. Studies have suggested that these phytochemicals, especially polyphenols, display high antioxidant properties, which help to reduce the risk of degenerative diseases, such as cancer and cardiovascular diseases. Several *in vitro* and *in vivo* studies have shown that Noni fruits have antioxidant, anti-inflammatory, anti-dementia, liver-protective, anticancer, analgesic, and immunomodulatory effects. In this study *M. citrifolia* fruit extracts were evaluated for its phytochemical profiling and anticancer activity. The extracts exhibited potential anticancer activity thus the methanolic extract of *M. citrifolia* can be used as novel anticancer agent in future.

Keywords: *Morinda citrifolia*, Anticancer, Phytochemical profiling, Liver cancer


https://doi.org/10.33745/ijzi.2022.v08i02.014

This is an Open Access Article licensed under a Creative Commons License: Attribution 4.0 International (CC-BY). It allows unrestricted use of articles in any medium, reproduction and distribution by providing adequate credit to the author(s) and the source of publication.

Introduction

Medicinal plants are precious part of the world flora. More than 80000 species out of the 2,500,000 higher plants on earth are reported to have at least some specific therapeutic value (Abbott, 1992). Traditional remedies derived from medicinal plants are non-toxic, effective, socially acceptable and economical. Medicinal plants play a significant role in providing primary health care services to people and serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine (Abdulla *et al.*, 2010). Since ancient times,
mankind has been dependent on plants for food, flavors, medicinal and many other uses. Ancient written records of many civilizations (i.e. Egyptian, Roman and Chinese) give strong evidence regarding use of medicinal plants. Ayurveda documents recorded the use of medicinal plants to cure many ailments. At present there are many well established herbal and plant medicine practices (Ayurvedic medicine in India) which are popular in many parts of the world. The World Health Organization (WHO) reported that 80% of people in the developing world use medicinal plants for their primary health care (Abha et al., 2013).

The use of herbal medicines is growing in developed countries, about 40% of compounds used in pharmaceutical industry are directly or indirectly derived from plants because the chemical synthesis of such compounds is either not possible and/or economically not viable (Abhijit and Yogini, 2015). Therefore, a large number of plant species (especially medicinal) are under threat of extinction because of their over exploitation. Human activity is the primary cause of risk for 83% of endangered plant species. Habitat destruction and loss are also a problem because they lead to the fragmentation of the remaining habitat resulting in further isolation of plant population. From another side during the last 10 years an intense interest has emerged in nutraceuticals (functional foods) in which phytochemical constituents can have long term health promoting or medicinal qualities (Abu Saleh et al., 2007).

Although the distinction between medicinal plants and nutraceuticals can sometimes be vague, a primary characteristic of the latter is that nutraceuticals have a nutritional role in the diet and the benefits to health may arise from long term use as foods (Ade and Rai, 2011).

*Morinda citrifolia* L. commonly known as “Noni”, is an important medicinal plant used by the ancient Indians to Polynesians since time immemorial. Among the different species of *Morinda, M. citrifolia* is widely exploited for commercial purposes due to its popularity in the health industry. *M. citrifolia* has been used as medicinal plants for preventing several cancers at the early stage of carcinogenesis and antioxidants activities of its fruit juice showed a dose-dependent *in vitro* effects against lipid peroxides and superoxides anion radical (SAR). The blockage of chemical carcinogen-induced DNA adducts and the strong antioxidant activity of fruit juice may contribute to the cancer preventive activity of *M. citrifolia* at the early stages of chemical carcinogenesis (Nualsanit et al., 2012).

Different parts of *M. citrifolia* plant have been the subject of medical researches investigating the plant’s effects on health. Few published *in vivo* and *in vitro* studies indicate that this plant exhibit the great use in alternative medicine for 80 various illnesses such as arthritis, diabetes, high blood pressure, muscle aches and pains, menstrual difficulties, headache, heart diseases, AIDS, gastric ulcer, sprains, mental depressions, senility, poor digestion, arteriosclerosis, blood vessel problems, drug addiction, and various cancers (Gupta et al., 2013).

During the last few decades, research has advanced on the role and involvement of free radicals, generated by the oxygen and nitrogen, in the pathogenesis of many degenerative ageing diseases with greater evidence regarding the number and contribution to their pathological pathologies of essential biological functions. The normal physiological production of reactive oxygen and nitrogen species and their elimination are finely balanced. The oxidation of lipids, DNA and proteins associated with cell damage can lead to an excess of oxidative stress (Vijayapandi et al., 2009).

There is evidence that a high antioxidants diet might have a beneficial effect on the basic ‘intrinsic’ ageing process and several disease processes linked with secondary age. It was proposed that increased fruit and vegetable consumption is linked with a reduced incidence of degenerative conditions such as cancer and atherosclerosis and that nutritional flavonoids can
clean up a spectrum of radical oxidative damage from DNA (Uma and Maheswari. 2014).

Generic cancer abnormalities usually impact the classes of the genes in general. Oncogenic cancer is generally activated in cells that provide these cells with new features, for example hyperactive growth and division, protection against the death of programmed cells, a lack of compliance with normal tissue borders and capacity to develop in a variety of tissue settings (Usha et al., 2010). The cancerous cell genes are then inactivated and the accurate DNA replication, cell cycle control, is failed.

Another frequent disease in the globe is liver cancer, likewise made up of metastasized malignant melanoma. Although many malignancies in the liver are secondary invasions (metastasis of other tumours), hepatocellular carcinoma (HCC) is the most prevalent kind of liver cancer (also named hepatoma, which is a misnomer because adenomas are usually benign)(Banerjee et al., 2006). A variety of HCC components and cholangio-carcinoma components also exist in this tumour. Most cases of cancer may be effectively treated when identified at the initial stage, and some of them can be healed by means of a combination of surgery, chemotherapy and radiation, depending upon the kind, location and stage of the treatment. However, once cancer has proceeded to a particular point, success is exceedingly low if it does not react to any traditional treatment (Nayak et al., 2011).

For the most part, one issue makes it difficult to eliminate cancer cells without affecting normal cells. However, substantial advances have been made in the creation of targeted therapeutic medicines that specially address identifiable tumour aberrations and reduce normal cell harm. But this strategy must be followed and many more research will be needed to see whether this approach can battle the disease successfully (Anwarul et al., 2010). In this study M. citrifolia fruit extracts were evaluated for its phytochemical profiling and anticancer activity.

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 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.

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

Culture of HepG-2 cells:
HepG-2 cell line was purchased from NCCS, Pune. Dulbecco’s modified Eagle’s medium (DMEM), trypsin, ethylenediamine tetraacetic acid, sodium bicarbonate, 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) were purchased from Gibco, Invitrogen Life Technologies and solvents like methanol and chloroform, were purchased from Hi Media.

Assessment of cytotoxicity by MTT assay:
The HepG-2 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 (20, 40, 60, 80 and 100 µg/ml) 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:

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

The percentage growth, percentage inhibition and 50% growth inhibition (IC\text{50}) values were calculated using a pre-programmed MS-Excel template.

**Statistical Analysis:**

The data of DPPH and MTT assay 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 all the solvents used in the present study (Aqueous, chloroform, and Methanol) showed the presence of carbohydrates, tannins, saponins, flavonoids, anthocyanin, glycosides, cardiac glycosides, terpenoids, triterpenoids and phenols. 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.

**Cytotoxicity of HepG-2 cells against *M. citrifolia* fruit extracts:**

The aqueous, chloroform and methanol extracts of *M. citrifolia* fruit exhibited cytotoxic effect against HepG-2 cells after 24 h exposure as determined by

### Table 1: Yield of solvent extracts of *Morinda citrifolia*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Solvents</th>
<th>Weight of dried extract (g)</th>
<th>Yield (%)</th>
<th>Colour</th>
<th>Consistency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aqueous</td>
<td>100</td>
<td>1.5</td>
<td>Dark brown</td>
<td>Paste</td>
</tr>
<tr>
<td>2</td>
<td>Chloroform</td>
<td>100</td>
<td>1.5</td>
<td>Dark brown</td>
<td>Paste</td>
</tr>
<tr>
<td>3</td>
<td>Methanol</td>
<td>100</td>
<td>2.5</td>
<td>Dark brown</td>
<td>Paste</td>
</tr>
</tbody>
</table>
Table 2: Qualitative phytochemical analysis of *Morinda citrifolia* fruit extracts

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

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

MTT assay. A concentration-dependent decrease in cell viability was observed for different extracts of *M. citrifolia* fruit on HepG-2 cells. A dose-dependent inhibition of HepG-2 cells was observed at different concentrations (20, 40, 60, 80 and 100 µg/ml) of *M. citrifolia* fruit extracts. The IC$_{50}$ value i.e., 50% growth inhibition of cell viability was obtained in 71.442 µg/ml concentration of methanol extract at 24 h, whereas, in aqueous and chloroform extracts, it was 93.505 µg/ml, 94.808 µg/ml, respectively (Table 3, Fig. 1). The inhibitory effect of methanol extract was significantly higher than that of the control. The control cells showed 100% viable cells because it has not undergone any treatment. The methanol extract showed better activity when compared to other solvent extracts and hence methanolic extract was used for further study. 71.442 µg/ml IC$_{50}$ concentration of methanol extract were used for further studies.
Table 3: Per cent cell viability of HepG-2 cells when treated for 24 h with various extracts of *Morinda citrifolia* fruit

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Aqueous extract</th>
<th>Chloroform</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 μg/ml</td>
<td>96.574±0.477 (-3.426)</td>
<td>97.274±0.022 (-2.726)</td>
<td>90.488±0.253 (-9.512)</td>
</tr>
<tr>
<td>40 μg/ml</td>
<td>89.352±0.192 (-10.648)</td>
<td>88.194±0.041 (-11.806)</td>
<td>78.304±0.046 (-21.696)</td>
</tr>
<tr>
<td>60 μg/ml</td>
<td>78.12±0.086 (-21.88)</td>
<td>75.574±0.066 (-24.426)</td>
<td>63.23±0.194 (-36.766)</td>
</tr>
<tr>
<td>80 μg/ml</td>
<td>62.876±0.404 (-37.124)</td>
<td>64.118±0.021 (-35.882)</td>
<td>40.102±0.068 (-59.898)</td>
</tr>
<tr>
<td>100 μg/ml</td>
<td>43.808±0.788 (-56.192)</td>
<td>45.05±0.01 (-54.95)</td>
<td>12.93±0.113 (-87.066)</td>
</tr>
<tr>
<td>IC50</td>
<td>93.505</td>
<td>94.808</td>
<td>71.442</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. of five individual observations; Values in parentheses are per cent change over control; - Denotes per cent decrease over control; *indicates significant value at P<0.05.

HepG-2 cells took 24 h to differentiate and to attach in basal conditions however, after the methanol extract treatment (71.442 μg/ml) the cell morphology was significantly altered, the control cells looked like ovoid and leaf like structure (Fig. 2). Whereas, the treated cells showed many disintegrated cells as well as dead cells (Figs. 3, 4).

*Cell viability by PI staining:*

The assessment of cell viability by PI staining provides a red fluorescence cell viability assay that is based on the simultaneous determination of live and dead cells with two probes that measure recognized parameters of cell viability—intracellular esterase activity and plasma membrane integrity. PI enters cells with damaged membranes and undergoes a 40-fold enhancement of fluorescence upon binding to nucleic acids, thereby producing a bright red fluorescence in dead cells (ex/em ~495 nm/~635 nm) (Fig. 5). PI is excluded by the intact plasma membrane of live cells. The determination of cell viability depended on the physical and biochemical properties of
Fig. 2: Anticancer activity of *M. citrifolia* aqueous extract against HepG-2 cells. A- Control HepG-2 cells; B- 93.505 μg/ml; C- 100 μg/ml.

Fig. 3: Anticancer activity of *M. citrifolia* methanol extract against HepG-2 cells. A- Control HepG-2 cells; B- 71.442 μg/ml; C- 100 μg/ml.

Fig. 4: Anticancer activity of *M. citrifolia* chloroform extract against HepG-2 cells. A- Control HepG-2 cells; B- 94.808 μg/ml; C- 100 μg/ml.

Fig. 5: PI staining of HepG-2 cells treated with *M. citrifolia* methanol extract. A- Control HepG-2 cells; B- 71.442 μg/ml; C- 100 μg/ml.
Discussion

Plants are the sources of phytochemicals which can be used for treating infectious diseases particularly in most of the developing countries of the world. The phytochemicals serve as natural antibiotics which help the human body to fight infections and microbial invasion (Muralidharan and Srikanth, 2010). Bioactive compounds are generally accumulated as secondary metabolites in all the plant cells but their concentration varies according to the plant part. The majority of these bioactive compounds are secondary metabolites which belong to groups of resins, fatty acids, tannins, flavonoids, steroids, alkaloids, phenol compounds, etc. Many studies have established that medicinal plants are sources of valuable nutrients and non-nutrient compounds, which exhibit antioxidant and antimicrobial properties to safeguard the human body against pathogens and cellular oxidation reactions as well (Nitteranon et al., 2011).

Apoptosis is the process of programmed cell death, which leads to characteristic cell change and death (Palu, 2009). Among cell apoptosis pathways, there are many proteins which play a critical role in the course of signal transduction. Caspases are the crucial mediators of apoptosis pathway, as frequently activated death proteases, whose function is to catalyze the specific cleavage of numerous cellular key proteins (Pandy and Khan, 2016). Among Caspases family, caspase-3 and caspase-8 are the two important members. Studies have shown that many herbal extracts induced cancer cell apoptosis mainly through up-regulating caspase-3 and caspase-8 expression. The Bcl2 family of proteins are key regulators of the apoptotic pathway. Bcl2 and BAX are the two important members in Bcl2 family, whose ratio determines whether a cell will undergo apoptosis or not (Pawlus and Kinghorn, 2007).

For many years, natural products have played an important role in health care and disease prevention. Evolution of multi drug resistance to antibiotics is greater cause of concern. The new generation of disease causing pathogens and mutations of existing microorganisms are responsible for human morbidity and mortality (Pawlus et al., 2005). Researchers and clinicians pay great attention to plant-derived secondary metabolites because of their antibiotic activity without conferring any antibiotic resistance. Hence, plant-based antimicrobials have widely been used as preventative and curative solutions against multi-drug resistant pathogens. Several plant species have already been widely reported showing potential medicinal properties. However, the emerging new infections, diseases and rapid evolution of pathogens urge the researchers for further exploration into nature for novel natural products (Potterat and Hamburger, 2007).

In the present study phytochemical screening showed the presence of carbohydrates, tannins, saponins, flavonoids, anthocyanin, glycosides, cardiac glycosides, terpenoids, triterpenoids and phenols. Methanolic extract showed the presence of all the secondary metabolites. Methanol extracts exhibited highly positive preliminary phytochemical tests. Collectively Methanolic extract showed the presence of all the secondary metabolites. Methanol extracts exhibited highly positive preliminary phytochemical tests. Collectively Methanol extract showed more positive results when compared to other extract such as aqueous and chloroform (Potterat, et al., 2007).

In present study, the changes in DPPH activity were significant with different concentrations of M. citrifolia fruit extracts. These fruit extracts exhibited good antioxidant potential when compared with that of L-Ascorbic acid standard. The calculated IC50 value for methanol extract was 71.442 μg/ml, aqueous extract was 93.505 μg/ml and for chloroform it was 94.808 μg/ml. The value showed that the methanol extract of M. citrifolia has high antioxidant potential than that of standard L-Ascorbic acid. Similar reports of antiradical power of different fractions of M. citrifolia was evaluated in vitro using DPPH assay.
Saludes et al. (2002) reported that the effective concentration (EC50) of alpha-pinene that gave 50% inhibition of the DPPH radical was found to be 310 μg/ml. Thus the findings of the above authors support the observations of present study. The methanol extract of M. citrifolia can exhibit prominent and potentially favourable actions on lessening the amount of precancerous lesions and enhance apoptosis in the liver cancer (Sarko and Pollack, 2002).

Conclusion

The antioxidant and anticancer potential of Morinda citrifolia fruit have been unraveled in the present study. Extraction of the endocarp of the fruit with aqueous, methanol and chloroform revealed that methanol gave maximum yield of extract. However, standardization of the solvents by MTT assay showed that only chloroform extract had significant effect on HepG-2 cells. Methanol extract has profound effect on HepG-2 cells. In toto, it can be concluded that extract of Morinda citrifolia has antioxidant and anticancer potential. Moreover, identification of active phytoconstituents in the extracts will pave a way for using this fruit as a natural cytotoxic agent against various cancers.

References


Paku AK. (2009) Noni (Morinda citrifolia L.) may improve memory: A mechanism involving its


