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Antibacterial, Antifungal and Tumor cell suppression potential of *Morinda citrifolia* fruit extracts

Sathish Kumar Jayaraman*, Muthu Saravanan Manoharan,
Seethalakshmi Illanchezian

Life Teck Research Centre, Vadapalani, Chennai, India

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Morinda citrifolia (noni) is indigenous to tropical countries and also considered as an important traditional folk medicine. *M. citrifolia* fruits extracted with three solvents (methanol, ethyl acetate and hexane) were tested *in vitro* for their antibacterial, antifungal and antitumor activity. Among the three solvents tested, methanol extract was active against all tested organisms with varied extents of antibacterial activity. Ethyl acetate extract was effective against most of the microorganisms tested except *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Hexane extract was ineffective against all tested microorganisms. Among the fungi tested, the maximum percentage of inhibition was observed against *Trichophyton mentagrophytes* with the extracts of methanol (79.3%) and ethyl acetate (62.06%). Nearly 50% inhibition was recorded against *Penicillium* sp., *Fusarium* sp. and *Rhizopus* sp. with methanol extract. None of the extracts were active against *Candida albicans* and *Aspergillus* species. The methanol extract showed maximum cytotoxicity on HEp2 cells followed by ethyl acetate extract. The overall results indicate promising baseline information for the potential uses of *M. citrifolia* fruit extracts in the treatment of infectious diseases and tumor.

Keywords: *Morinda citrifolia*; antibacterial; antifungal; antitumor; HEp2 cells.

INTRODUCTION

Herbal and natural products have been used in folk medicine for centuries throughout the world. There has been renewed interest in screening higher plants for novel biologically active compounds, particularly those that effectively intervene in human ailments (Mathivanan *et al.*, 2006). *Morinda citrifolia* (*Rubiaceae*), commercially known as noni, is indigenous to tropical countries and is considered as an important traditional folk medicine. *M. citrifolia* have a history of use in Polynesian traditional medicine for the treatments of infectious diseases (Locher *et al.*, 1995). The indigenous tribes of Australia used the ripe fruits of *M. citrifolia* for treatment of respiratory infections (Peerzada *et al.*, 1990). It has been reported to have a broad range of therapeutic and nutritional values (Whistler, 1992). There is a great demand for its fruit juice in treatment for different kinds of illnesses such as arthritis, diabetes, muscle aches, menstrual difficulties, heart disease, cancers,

gastric ulcer, blood vessel problems, and drug addiction (Wang *et al.*, 2002). A number of major components have been identified in the noni plant, which includes scopoletin, octoanoic acid, terpenoids, alkaloids, anthraquinones, b-sitosterol, carotene, flavone glycosides, linoleic acid, alizarin, acubin, L-asperuloside, caproic acid, caprylic acid, ursolic acid, rutin, and proxeronine (Levand and Larson, 1979; Farine *et al.*, 1996; Moorthy and Reddy, 1970 and Singh and Tiwari, 1976).

Even though tens of thousands of antimicrobial compounds exist, the ability of microbes to develop resistance to even the most powerful antimicrobial compounds is amazingly rapid (Gerson *et al.*, 2006). In order to keep pace with this ever increasing need for new antimicrobials, it is imperative that new compounds be discovered. The methanol extracts from *M. citrifolia* leaves was reported to have antibacterial activity (Nakanishi *et al.*, 1965). The fruit juice of *M. citrifolia* contains a polysaccharide-rich substance, which has been reported to have antitumor activity in the Lewis lung peritoneal carcinoma model (Hirazumi *et al.*, 1994).

*Corresponding author:
Sathish Kumar Jayaraman,
Life Teck Research Centre,
Vadapalani, Chennai – 600026, India
Email: jsathishkumar85@gmail.com

The present study was initiated with the aim of investigating the antimicrobial and tumor cell suppression potential of fruit extracts of *M. citrifolia* which might have possible application in the treatment of infectious diseases and tumor.

MATERIALS AND METHODS

Test organisms

Bacillus subtilis, *Staphylococcus aureus*, *Lactobacillus lactis*, *Streptococcus thermophilus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, *Vibrio harveyi*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Salmonella paratyphi A*, *Aeromonas hydrophila*, *Vibrio cholerae*, *Chromobacterium violaceum* and *Enterobacter faecalis* were used for testing antibacterial activity.

Candida albicans, *Aspergillus niger*, *Trichophyton mentagrophytes*, *Penicillium sp.*, *Fusarium sp.*, *Aspergillus fumigatus*, *Mucor sp.*, *Rhizopus sp.* and *Aspergillus flavus* were used for testing antifungal activity.

Preparation of fruit extract

The fresh fruits of *M. citrifolia* were purchased from Health India laboratories, Chennai, a unit of Indian Noni Research Foundation. The fruits were chopped into pieces and dried at room temperature for 24 hr. The air dried fruits were kept at 40 °C in hot air oven for 24 hr to remove moisture content. The completely dried fruits were ground into powder by using mortar and pestle. About 60 g of the dried fruit powder was mixed in the ratio (w/v) of 1:5 with each of the solvents namely methanol, ethyl acetate, and hexane (Qualigens, India). The extraction was carried out in a shaker water bath at 40 °C for 48 hr. The extracts were filtered through Whatmann No.1 filter paper. The extracts were concentrated to dryness and dissolved in 0.25% Dimethyl Sulphoxide (DMSO, Merck) to the concentration of 100 mg / mL.

Antibacterial assay

Agar well bioassay was employed for testing antibacterial activity of *Morinda citrifolia* fruit extracts (Linday, 1962). About 0.5 mL of 24 hr old culture of test organisms were seeded onto Mueller Hinton agar (HiMedia, India) plate and uniformly spread with spreader. Wells (5 mm)

were made on the plate with sterile cork borer. The fruit extract was introduced into the well and the plates were incubated at 37 °C for 24 hr. The antibacterial activity of fruit extract was determined by measuring the diameter of the inhibition zone. Controls were maintained with DMSO only. The antibacterial assay was performed in triplicates.

Antifungal assay

Potato dextrose agar (HiMedia) was prepared and 1 mL of fruit extract was added to 100 mL of medium. After solidification a loop full of culture was placed on the centre of the plate. Controls were maintained with DMSO only. All the plates were incubated at 25 °C for 4 days (Linday, 1962). The growth of the fungal cultures were measured and compared with their respective control plates. The antifungal assay was performed in triplicates.

Antitumor assay

The antitumor assay was performed on human laryngeal epitheloma (HEp2) cells obtained from King Institute of Preventive Medicine, Chennai, India. The cells were grown in 24 well plate (Falcon) in Eagle's Minimum Essential Medium (HiMedia) supplemented with 10% fetal bovine serum (Gibco Laboratories) and antibiotics (streptomycin, penicillin-G, kanamycin, amphotericin B, HiMedia). The cell suspension (10^5 cells / mL) was seeded in every well and incubated at 37 °C for 48 hr in 5% CO₂ for the formation of confluent monolayer. The monolayer of cells in 24 well plate was exposed to various dilutions of the fruit extract. The cell viability was measured using MTT assay as described by Mosmann (1983) using MTT (5 mg / mL) and DMSO. Cell control was maintained throughout the experiment and the assay was performed in triplicates.

RESULTS

The yield of *M. citrifolia* fruit extracts using methanol, ethyl acetate and hexane as solvents were 22.73, 1.9 and 0.11% respectively, on dry weight basis. The *in vitro* antibacterial activity of methanol, ethyl acetate and hexane extracts of *M. citrifolia* fruits are shown in Table 1 and Fig. 1. Among the three solvent extracts tested, methanol extract showed maximum inhibitory potential against all tested microorganisms followed by ethyl acetate and hexane

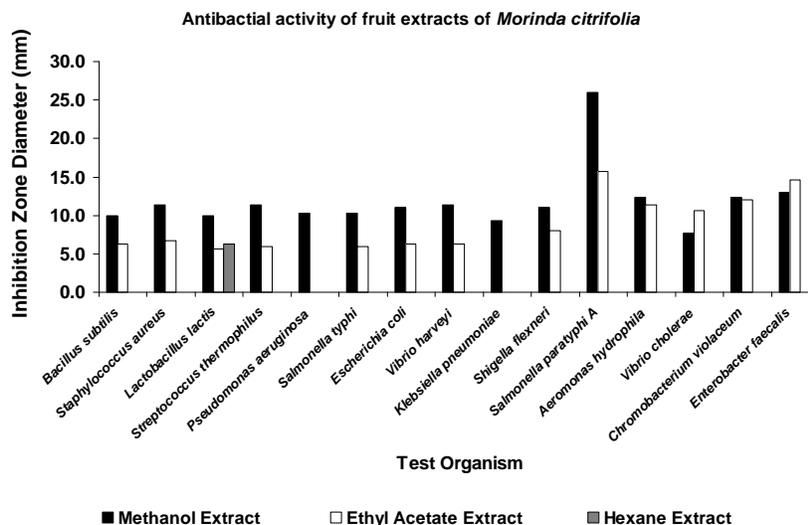


Figure 1: Antibacterial activity of fruit extracts of *Morinda citrifolia* (Test organism Vs Inhibition zone diameter produced by extracts tested)

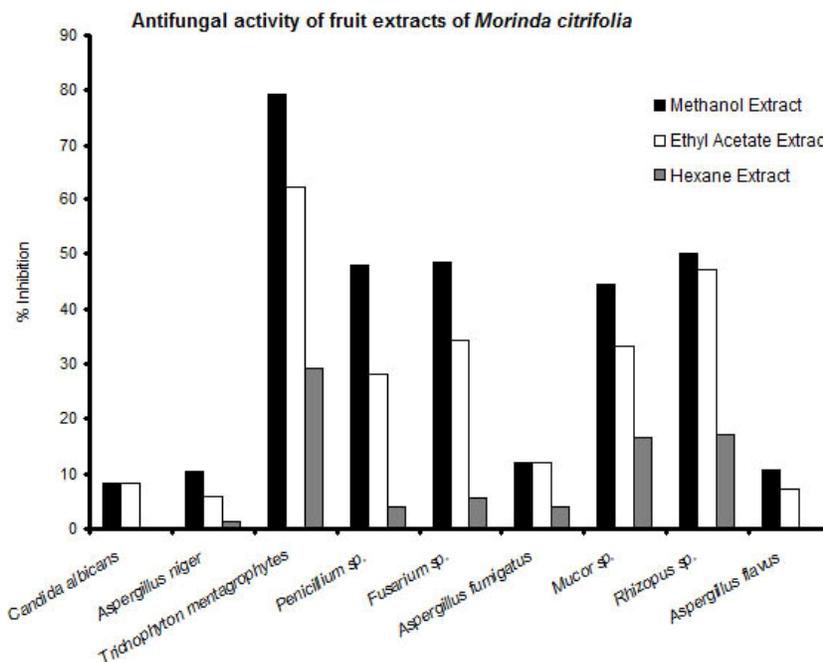


Figure 2: Antifungal activity of fruit extracts of *Morinda citrifolia* (Test organism Vs % Inhibition of fungal culture produced by extracts tested)

respectively. Methanol extract was active against both gram positive and gram negative organisms with varied extents of antibacterial activity. The maximum inhibition was recorded against *Salmonella paratyphi A* with the extracts of methanol (27 mm) and ethyl acetate (16 mm). Ethyl acetate extract was effective against most of the microorganisms tested except *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The ethyl acetate extract recorded

significant activity against *Salmonella paratyphi A* (16 mm), *Chromobacterium violaceum* (12 mm), *Aeromonas hydrophila* (11 mm). Hexane extract was ineffective against both gram positive and gram negative bacteria tested.

The antifungal activity of methanol, ethyl acetate and hexane extracts of *M. citrifolia* fruits are shown in Table 2 and Fig. 2. Among the organisms tested, maximum percentage of

Table 1: Antibacterial activity of fruit extracts of *Morinda citrifolia* against gram positive and gram negative bacteria

Microorganism	Methanol extract (in mm)	Ethyl acetate extract (in mm)	Hexane extract (in mm)
<i>Bacillus subtilis</i>	10.0 ± 1.0	6.3 ± 0.6	-
<i>Staphylococcus aureus</i>	11.3 ± 0.6	6.7 ± 0.6	-
<i>Lactobacillus lactis</i>	10.0 ± 0.0	5.7 ± 0.6	6.3 ± 0.6
<i>Streptococcus thermophilus</i>	11.3 ± 0.6	6.0 ± 1.0	-
<i>Pseudomonas aeruginosa</i>	10.3 ± 0.6	-	-
<i>Salmonella typhi</i>	10.3 ± 0.6	6.0 ± 0.0	-
<i>Escherichia coli</i>	11.0 ± 0.0	6.3 ± 0.6	-
<i>Vibrio harveyi</i>	11.3 ± 0.6	6.3 ± 0.6	-
<i>Klebsiella pneumoniae</i>	9.3 ± 0.6	-	-
<i>Shigella flexneri</i>	11.0 ± 0.0	8.0 ± 0.0	-
<i>Salmonella paratyphi A</i>	26.0 ± 1.0	15.7 ± 0.6	-
<i>Aeromonas hydrophila</i>	12.3 ± 0.6	11.3 ± 0.6	-
<i>Vibrio cholerae</i>	7.7 ± 0.6	10.7 ± 0.6	-
<i>Chromobacterium violaceum</i>	12.3 ± 0.6	12.0 ± 1.0	-
<i>Enterobacter faecalis</i>	13.0 ± 1.0	14.7 ± 0.6	-

Note: There was no inhibition found in control.

Table 2: Antifungal activity of fruit extracts of *Morinda citrifolia*

Microorganism	Control (in mm)	Methanol extract (in mm)	% inhibition	Ethyl acetate extract (in mm)	% inhibition	Hexane extract (in mm)	% inhibition
<i>Candida albicans</i>	12 ± 0.0	11 ± 0.0	8.3	11 ± 0.0	8.3	12 ± 0.0	0
<i>Aspergillus niger</i>	67 ± 1.0	60 ± 0.0	10.5	63 ± 0.6	5.9	66 ± 0.6	1.4
<i>Trichophyton mentagrophytes</i>	58 ± 0.0	12 ± 0.0	79.3	22 ± 0.6	62.06	41 ± 0.6	29.3
<i>Penicillium</i> sp.	25 ± 0.6	13 ± 0.6	48.0	18 ± 0.0	28	24 ± 0.0	4
<i>Fusarium</i> sp.	35 ± 1.0	18 ± 0.6	48.5	23 ± 0.6	34.2	33 ± 1.0	5.7
<i>Aspergillus fumigatus</i>	25 ± 0.0	22 ± 0.6	12	22 ± 0.6	12	24 ± 0.6	4
<i>Mucor</i> sp.	90 ± 1.0	50 ± 0.6	44.4	60 ± 1.0	33.33	75 ± 0.6	16.6
<i>Rhizopus</i> sp.	70 ± 0.0	35 ± 1.0	50	37 ± 0.6	47.1	58 ± 1.0	17.1
<i>Aspergillus flavus</i>	28 ± 0.6	25 ± 0.6	10.7	26 ± 0.0	7.14	28 ± 0.0	0

inhibition was observed against *Trichophyton mentagrophytes* with the extracts of methanol (79.3%) and ethyl acetate (62.06%). Nearly 50% inhibition was recorded against *Penicillium* sp., *Fusarium* sp. and *Rhizopus* sp. with methanol extract. Hexane extract exhibited the least percentage of inhibition against all fungal

cultures. None of the extracts showed significant activity against *Candida albicans* and *Aspergillus* species.

The tumor cell suppression potential of *M. citrifolia* fruit extracts with methanol, ethyl acetate and hexane as solvents on HEP2 cells

was recorded. The cytotoxicity of three solvent extracts of *M. citrifolia* fruits on HEP2 cells was measured using MTT assay as shown in Fig. 3. The methanolic extract showed maximum cytotoxicity on HEP2 cells followed by ethyl acetate extract. The hexane extracts showed no activity on HEP2 cells.

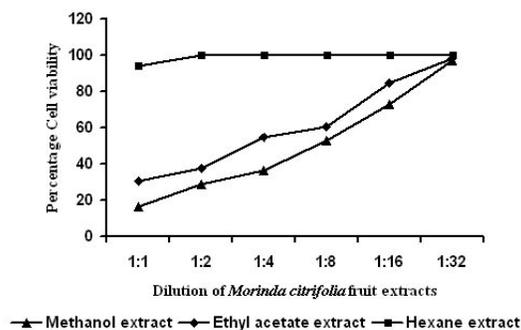


Figure 3: Cytotoxicity of *Morinda citrifolia* fruit extracts with methanol, ethyl acetate and hexane as solvents on human laryngeal epitheloma cells (HEP2)

DISCUSSION

The yield of *M. citrifolia* fruit extracts was higher in methanol, compared to ethyl acetate and hexane. The yield obtained decreased with decrease in polarity. Since methanol has high polarity, it could dissolve both the polar and non polar compounds in it.

The methanol extract inhibited both gram positive and gram negative bacteria significantly, compared to ethyl acetate and hexane extract. Similar to the results reported by Isami *et al.* (2007), the methanol extract was more active than ethyl acetate extract against tested microorganisms. The maximum and minimum antibacterial activity was recorded for methanol extract against *Salmonella paratyphi A* (27 mm) and *Vibrio cholerae* (8 mm) respectively. Ethyl acetate extract inhibited *Enterobacter faecalis* (15 mm) and *Vibrio cholerae* (11 mm) to higher extent when compared to methanol extract of *M. citrifolia* fruits. The hexane extract was not significant against any of the microorganisms tested, which may be due to poor solubility of active compounds in it.

The methanol extract of *M. citrifolia* fruits remarkably inhibited the mycelial growth of all the fungi tested as compared to control. Methanol extract was more potent against tested fungal cultures, followed by ethyl acetate extract.

Hexane extract showed least inhibitory potential against all fungal cultures tested. Nearly 50 % inhibition was recorded against *Penicillium sp.* (48.0%), *Fusarium sp.* (48.5%), and *Rhizopus sp.* (50%) for methanol extract.

Among the gram positive microorganisms tested with ethyl acetate extract, maximum activity was recorded against *Staphylococcus aureus* (7 mm) and among the fungi tested; maximum percentage of inhibition was recorded against *Trichophyton mentagrophytes* (62%). This observation is in agreement with Isami *et al.* (2005) who reported that ethyl acetate extracts of *M. citrifolia* fruits showed significant activity against *Trichophyton mentagrophytes* and *Staphylococcus aureus* when compared to other tested organism. But in this study, the maximum activity was recorded for methanol extract against *Trichophyton mentagrophytes* (79.3 %) and *Staphylococcus aureus* (11 mm). None of the extracts were significant against *Candida albicans*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger*.

The methanol extracts of *M. citrifolia* fruits inhibited nearly 50 % of HEP2 cells upto 1:8 dilution of the crude extract. Arpornsuwan and Punjanon (2006) reported that the methanolic extract of *M. citrifolia* fruit was much more effective on breast cancer cells and neuroblastoma cells. Similarly, the alcohol extract of noni fruit at various concentrations was reported to inhibit the production of tumor necrosis factor-alpha (TNF- α), which is an endogenous tumor promotor (unpublished data).

The antibacterial, antifungal and antitumor activity was at its peak in methanolic extract indicating that most of the active components are extracted with methanol. The overall results indicate promising baseline information for the potential uses of the methanol and ethyl acetate extracts of *M. citrifolia* fruit in the treatment of infectious diseases and tumor.

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