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Original Research Article

Antimicrobial activities of hydroethanolic extract of *Morinda citrifolia* fruit

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A B S T R A C T

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. One such important fruit is *Morinda citrifolia. L,* a member of Rubiaceae family which is commonly known as Noni and is used in traditional medicine as a remedy for various diseases. Hydroethanolic extract of *Morinda citrifolia* were screened for their antimicrobial activity using Disc Diffusion method. The antibacterial activity of hydroethanolic extract of *M. citrifolia* at 100µg/ml concentration was found to be more effective against *Klebsiella pneumoniae* than other tested organisms. Standard drug Ciprofloxacin exhibited maximum antibacterial activity at 5µg/ml. The antifungal activity was observed against all the tested fungi at 100µg of hydroethanolic extract. However, it was much effective against *Aspergillus fumigatus* at the concentration of 20µg/ml. From the observations made *Morinda* fruit extract exerted concentration dependant antimicrobial activity against tested bacteria and fungi. *Morinda citrifolia* constitutes bio active principles which are responsible for the antibacterial and antifungal activity including other medicinal values and physiological activities.

Keywords

Morinda citrifolia,
Hydroethanolic extract,
Noni,
Disc Diffusion method

Introduction

The search of new antibacterial and antioxidant agents from natural sources has intensified in response to the limitations of currently available therapy. Plants produce diverse range of bioactive molecules making them rich sources of different types of potential drugs (Walton *et al.,* 1999, Ghasemzadeh *et al.,* 2010). Herbal plants are reservoirs of secondary metabolites which are being exploited as source of bioactive substance for various pharmacological purposes (Adefuye and Ndip, 2013).

Medicinal plants have immensely contributed to sustenance of human health since the time immemorial. Microorganisms cause a number of deleterious diseases in man, animal and plants. Synthetic drugs and antibiotics do not completely cure these diseases because the microorganisms develop resistance against these compounds (Conlon *et al.,* 2003). Several hundreds of plants represent good sources of therapeutic agents and are used traditionally for different purposes including treatment of bacteria, fungi and viral infections (Bessong

et al., 2006). *Morinda citrifolia* is one of such plant with wide ethanomedicinal use.

Morinda citrifolia belongs to the family Rubiaceae, commonly known as Noni and it is a most popular drug in ayurvedic medicine. It is a tropical and subtropical plant grown in the pacific islands and has been used to treat a broad range 2000 diseases approximately (Mc Clatchey, 2002). Commercial noni juice and encapsulated noni powder have become popular in Asia, North America and Europe. Various unsubstantiated claims are currently made for noni products, such as functions as an immune system stimulant, anticancer agent, menstrual cycle regulator and blood cleanser (Nelson, 2002).

Biological compounds such as glycosides, polysaccharides, iridoids, alkaloids, lignans, trisaccharide fatty acid esters, anthroquinones, scopoletin, morindin, vitamins and minerals have been isolated from noni fruits, roots and leaves (Su *et al.*, 2005). The present study was carried out to evaluate the antimicrobial activities of hydroethanolic extract of *Morinda citrifolia* fruit.

Materials and Methods

Plant Material

The fruits of *Morinda citrifolia* were collected from the local areas around Chennai and Coimbatore and authenticated by Botanical Survey of India (BSI) in 'Tamil Nadu Agriculture University', Coimbatore. A voucher specimen (No: BSI/SRC/5/23/2012-13/Tech 44) has been deposited at the herbarium of the Botany Department. The samples were washed with running tap water and separated before being chopped into pieces. They were oven dried at 45°C for 2 days and ground to powder.

Preparation of extracts

The powdered material of *Morinda citrifolia* was extracted with two different solvents like 50% ethanol, water in two different Soxlet extractors exhaustively for 20–24 hours. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40–50°C). The dried extracts obtained were used in this study.

Microorganisms

The following bacterial strains were employed in the screening: *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniaee.* In the antifungal screening the following fungi were tested: *Aspergillus niger, Aspergillus fumigatus, Monoscus purpureus, Candida albicans, Monoscus rubber.*

Antibacterial activity preparation of inoculum

The inoculums for the experiment were prepared in fresh Nutrient broth from preserved slant culture. The inoculums were standardized by adjusting the turbidity of the culture to that of McFarland standards. The turbidity of the culture may be adjusted by the addition of sterile saline or broth (if excessive or by further incubation to get required turbidity (Leonard Jarrett *et al.*,).

Preparation of Sterile swabs

Cotton wool swab on wooden applicator or plastics were prepared and sterilized by autoclaving or dry heat (only for wooden swabs) by packing the swabs in culture tubes, papers or tins etc.

Experiment

The standardized inoculums are inoculated in the plates prepared earlier (aseptically) by dipping a sterile in the inoculums removing the excess of inoculums by passing by pressing and rotating the swab firmly against the side of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60^0C after each application. Finally pass the swab round the edge of the agar surface. Leave the inoculums to dry at room temperature with the lid closed.

Each Petri dish is divided into 2 parts, in 1 part extract sample disc (100mcg), (discs are soaked overnight in extract solution) and another part for STD Ciprofloxacin 5µg, are placed in each part with the help of sterile forceps. Then Petri dishes are placed in the refrigerator at 4^0C or at room temperature for 1 hour for diffusion. Incubate at 37^0C for 24 hours. Observe the zone of inhibition produced by different Antibiotics. Measure it using a scale or divider or venire calipers and record the average of two diameters of each zone of inhibition.

Anti fungal activity preparation of inoculum

The inoculums for the experiment were prepared in fresh sabouraud's broth from preserved slant culture. The inoculums were standardized by adjusting the turbidity of the culture to that of McFarland standards. The turbidity of the culture may be adjusted by the addition of sterile saline or broth [if excessive or by further incubating to get required turbidity (Leonard Jarrett *et al.,*)].

Preparation of sterile swabs

Cotton wool swab on wooden applicator or plastics were prepared and sterilized by autoclaving or dry heat (only for wooden swabs) by packing the swabs in culture tubes, papers or tins etc.

Minimum Inhibitory Concentration preparation of test drug

Serial 2-fold dilutions of the test antimicrobial agent were made in 1ml of Muller Hinton Broth. Series of 10–15 dilutions to final concentrations of 100-1.56µg/ml are prepared.

Preparation of inoculum:

Overnight culture are grown at 37^0C Kirby-Bauer procedure and diluted to Muller Hinton Broth. This overnight culture was diluted to 10^{-2}. The sterile tubes were labeled 1–8 and 8^{th} tube was taken as control. 1ml of Muller Hinton Broth was transferred to all tubes except 6^{th} & 7^{th}. 0.1ml of broth was transferred to 6^{th} & 7^{th} tubes. 1ml of drug solution was added to 1^{st} tube and mixed well. From the 1^{st} tube 1ml of solution was transferred to the 2^{nd} tube and was repeated up to 6^{th} tube. From the 6^{th} tube 0.5ml of solution was taken and transferred to 7^{th} tube. 0.1ml of culture was added to all the test tubes. All the tubes were incubated at 37^0C for 18–24hrs. After incubation observe the turbidity or OD value by spectrophotometer method.

Results and Discussion

Knowledge about the traditional medicines and their exercise has been accomplished and the same have been handed over to the descending generations orally or in written. The traditional medicines were being used in prevention, diagnosis and treatment of physical and mental ailments based only on experience and observations. Modern science has taken many positive steps to

bring them in frontier of science and technology through research.

Antibacterial activities of hydroethanolic extract of *Morinda citrifolia* fruits

Hydroethanolic extract of *Morinda citrifolia* fruit was found to be competent to inhibit the growth of both Gram positive (*Bacillus subtilis*, and *Staphylococcus aureus*) and Gram negative bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) (Table 1).

The highest antibacterial activity of 12mm Zone of inhibition in *Klebsiella pneumoniae* and moderate activity of 11mm Zone of inhibition in *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains were noticed. The least activity was recorded against *Bacillus subtilis* and *Escherichia coli* (10mm) respectively. Our observations were in accordance with the findings of previous results which proved the antimicrobial activity of hydroethanolic extract of *Morinda citrifolia* which might be due to the presence of phytonutrients.

Gram negative bacteria were more susceptible to *M. citrifolia* fruit extract than gram positive bacteria. Membrane accumulator mechanism might play important role behind this perception (Basri *et al.,* 2005, Vital *et al.,* 2009). Earlier reports have demonstrated the antibacterial activity of leaf, stem and fruit of *Morinda citrifolia* against wide spectrum of gram positive and gram negative bacterial strains (Esath *et al.*, 2012). In the present study, *M. citrifolia* fruit was screened for antibacterial effect and the results obtained proved that moderate activity was seen in all the tested bacteria when compared with the standard Ciprofloxacin. Minimum inhibitory concentration (MIC) values favorably ensure that *M. citrifolia* could be recommended to treat infectious disease (Usha *et al.,* 2010). Our results were in accordance with the previous studies which suggest that tannins and flavonoids might enable the extract to overcome the barrier in bacterial cell wall (Anyasor *et al.,* 2011).

Selvam *et al* (2009) studied the antimicrobial activity of acetone, chloroform, ethanol and methanol extract of *M. citrifolia* fruit against *E. coli, Proteus vulgaris and S. aureus.* The samples exhibited considerable activity against *E. coli* and *S. aureus* strains which are similar to our study. Rivera *et al.,* (2011) also revealed the antibacterial activity of *M. citrifolia* fruit juice against *Mycoplasma pneumoniae, Mycoplasma penetrans* and *Mycoplasma fermentans* which supported our findings. The petroleum ether and alcoholic extract of *Morinda citrifolia L*. (Noni) leaves were screened for antimicrobial properties against *E. coli, B. subtilis* and *S. aureus* by Khuntia *et al.,* (2012) who confirmed that 10 mg/ml extract showed maximum growth inhibition against *E. coli* (2.4 cm). Similarly various extracts of *Adiantum capillus-veneris* was recently investigated for antibacterial efficacy and potent antibacterial effect against a number of strains such as *E. coli, Pseudomonas, Citrobacter, Klebsiella, Proteus, Vibrio, Shigella, Salmonella, S. aureus* and *Providencia* was recorded by Muhammad *et al.,* (2014). The antibacterial study revealed that *M. citrifolia* fruit extract possesses the antibacterial activity against both the Gram positive and Gram negative bacterial strains.

Anti Fungal activity of Hydroethanolic extract of *Morinda citrifolia* fruits

The antifungal activity of the *M. citrifolia* hydroethanolic fruit extract was determined against five fungi species such as *Aspergillus niger, Aspergillus fumigatus,*

Monoscus purpureus, Candida albicans and *Monoscus rubber* and are recorded in Table 2. Maximum zone of inhibition was observed in hydroethanolic fruit extract against *Aspergillus fumigatus* (12 mm) and *Monoscus purpureus* (12 mm). Minimum zone of inhibition was observed with Noni fruit against *Aspergillus niger* (9mm) and *Candida albicans* followed by *Monoscus rubber* which was recorded to be 11mm. The results were compared with the standard drug Fluconazole. The *Morinda citrifolia* fruit extract showed more or less same activity against all tested organisms. Our observations are in line with Sathish Kumar *et al.,* (2008) who reported the maximum antifungal activity for methanolic extract against *Trychophyton mentagrophytes.*

M. citrifolia fruit holds a wide range of therapeutic effects including *in vitro* anticandidal activity due to the presence of acubin, L-asperuloside, alizarin, scopoletin and other anthraquinones (Banerjee *et al.,* 2006). Earlier study of Khuntia *et al.,* (2012) proved that the ethanolic and petroleum ether extract of *M. citrifolia* fruit showed potent antifungal activity. Our findings are in corroboration with the observation made by Abu Mejdad who proved that highest antifungal activity was observed with ethanolic extract of *Qurecus egilops* against tested fungal strains.

The results clearly demonstrated that *Morinda citrifolia* fruit extracts possess substantial antifungal properties and used in the treatment of fungal infections.

Minimum inhibitory concentration of *Morinda citrifolia* extract

Minimum inhibitory concentrations of the hydroethanolic extract of *Morinda citrifolia* fruit against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Candida albicans, Aspergillus niger, Monoscus rubber, Monoscus purpureus,* and *Aspergillus fumigatus* organisms have been illustrated in table 3 and 4. Minimum concentration at which microbial growth was absent was determined in this study.

The minimum inhibitory concentration exhibited for *Morinda* fruit extract against *Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans, Aspergillus niger, Monoscus rubber, Monoscus purpureus,* and *Aspergillus fumigatus* were found to be 50 µg. Minimum inhibitory concentration against *Escherichia coli* and *Bacillus subtilis* showed maximum MIC at 100 µg/ml. Minimum inhibitory concentration ranges from 50–100µg/ml (Table 4). Growth of tested bacteria was not found in the sub-culture of the tubes above the minimum inhibitory concentration (MIC) in the tested fruit extract. Similar results were observed by Esath who reported the inhibitory effect of *Morinda citrifolia* against the growth of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus sp., Klebsiella pneumoniaee, Shigella flexneri, Proteus mirabilis, Pseudomonas diminuta, Pseudomonas fluorescens* and *Enterobacter cloacae* (Esath *et al.,* 2012).

MIC values tend to vary against organism as well as the plant extracts chosen. Maji *et al* (2010) evaluated *Borreria hispida* (Linn) for antibacterial activity against various organisms, and MIC of methanolic extract was recorded to range from 50 to 250mg/mL. Moreover higher MIC values ranging from 0.125 to 32 mg/mL were also reported against at least one of the test microorganisms. Bioactive compounds such as flavonoids, alcohols, aldehydes, aromatic compounds, fatty acid methyl esters, terpenoids, phenolics, and steroids were

postulated for antibacterial activity presented by plant extracts including leaf, stem, root and flowers (Farina *et al.*, 2014). Our study was in par with the results obtained by Mathew *et al.*, (2014) who screened for minimum inhibitory concentration of leaf extracts against wide spectrum of bacterial species which can be used as an alternative to orthodox antibiotic in the treatment of various diseases caused due to infection of microorganisms.

Table.1 Antibacterial activity of hydroethanolic extracts of *Morinda citrifolia* fruit

Name of the microorganisms	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
Standard Ciprofloxacin(5µg)	15	22	18	21	16
Fruit extract (100µg)	10	11	10	11	12

Table.2 Antifungal activity of hydroethanolic extracts of *Morinda citrifolia* fruit

Name of the microorganisms	*Aspergillus niger*	*Aspergillus fumigatus*	*Monoscus purpureus*	*Candida albican*	*Monoscus rubber*
Standard Fluconazole (20µg)	**10**	**21**	**20**	**12**	**21**
Study of extract (100µg)	**09**	**12**	**12**	**09**	**11**

Table.3 Inhibitory effect of *Morinda citrifolia* fruit extract against microorganisms in µg/ml

S.No	**Pathogens**	**200 µg/ml**	**100 µg/ml**	**50 µg/ml**	**25 µg/ml**	**12.5 µg/ml**	**6.25 µg/ml**	**3.12 µg/ml**
1.	*Escherichia coli*	-	-	+	+	+	+	+
2.	*Klebsiella pneumoniae*	-	-	-	+	+	+	+
3.	*Pseudomonas aeruginosa*	-	-	-	+	+	+	+
4.	*Bacillus subtilis*	-	-	+	+	+	+	+
5.	*Staphylococcus aureus*	-	-	-	+	+	+	+
6.	*Candida albicans*	-	-	-	+	+	+	+
7.	*Aspergillus niger*	-	-	-	+	+	+	+
8.	*Monoscus rubber*	-	-	-	+	+	+	+
9.	*Monoscus purpureus*	-	-	-	+	+	+	+
10.	*Aspergillus fumigatus*	-	-	-	+	+	+	+

Table.4 Minimum Inhibitory Concentration of *Morinda citrifolia* fruit extract against microorganisms

S.No	Pathogens	MIC
1.	*Escherichia coli*	100 μg/ml
2.	*Klebsiella pneumoniae*	50 μg/ml
3.	*Pseudomonas aeruginosa*	50 μg/ml
4.	*Bacillus subtilis*	100 μg/ml
5.	*Staphylococcus aureus*	50 μg/ml
6.	*Candida albicans*	50 μg/ml
7.	*Aspergillus niger*	50 μg/ml
8.	*Monoscus rubber*	50 μg/ml
9.	*Monoscus purpureus*	50 μg/ml
10.	*Aspergillus fumigatus*	50 μg/ml

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