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Antibacterial Activity in Solvent Extract of Different Parts of *Morinda citrifolia* Plant

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Abstract
During the present study the antibacterial activity of *Morinda citrifolia* leaf, fruit and seed extract was assessed *in-vitro* by using disc diffusion method. A total of five different accessions of *M. citrifolia* plants were randomly selected and screened for antibacterial activity against five different bacterial pathogens. Overall analysis of the antibacterial activity of various extracts revealed that the best inhibitory activity was produced by the seed extract (12.23 mm) compared to the leaf and fruit extract. *E.coli* and the *Pseudomonas spp* were inhibited by all the extracts; however, *Salmonella spp., Staphylococcus aureus* and *Klebsiella spp* were not inhibited by all the extracts. The analysis of antibacterial activity of all the accessions revealed that the best antibacterial activity was produced by accession 5 followed by accession 1, 2, 3 and 4 respectively.

Keywords: Antibacterial activity; *Morinda citrifolia*; Solvents

1. INTRODUCTION

The *Morinda citrifolia* L. is a small evergreen tree found growing in open coastal region at sea level and in forest. It belongs to the family Rubiacae. The plant is commonly known as Noni, Indian mulberry, Ba Ji Tian, nano or nonu, nahu, cheese fruit etc in different parts of the world (Whistler). The roots, stem, bark, leaves, flowers and fruits of the plant are involved in various combinations in almost 40 known and recorded herbal medicines. More than 160 neutraceutical compounds have been identified from the plant (Solomon, 1999, 2000).

In the recent years attention has been diverted towards the search for new novel compounds from the plant, animals and microbes. Due to the increasing trend of multidrug resistance the study has been concentrated on newer antimicrobial compounds from the plant origin. A number of plants have been identified with the properties of antimicrobial activity. Research has also been carried out on various aspects of *M.citrifolia* L. However, scientific evidence of the benefits of the noni fruit juice is very limited but there are some anecdotal evidences for treatment of influenza (Hirazumi *et al*, 1994,1996). It was observed that extracts of *M.cirifolia* L. exhibited significant antibacterial and antifungal activity against various strains of bacteria and fungi (Gerson, 2002). The extract from the ripe fruit was shown to have moderate antibacterial properties against *S. typhosa, S. montevideo, S. schottmuelleri, Sh. paradys* BH (Bushnell *et al*, 1950). However, there is very little information available on antibacterial and antifungal activity of different parts of the plants using various solvents extract. Therefore, the present study was designed to study the antibacterial properties of different accession of the *M. citrifolia* L. plant to select the best accession for further propagation.

2. MATERIALS AND METHODS

2.1. Microorganisms

A total of five different accessions of *M. citrifolia* plants were randomly selected and screened for antibacterial activity against five different bacterial pathogens viz. *Salmonella spp., Pseudomonas spp., Staphylococcus aureus, Klebsiella spp.,* and *E.coli.*

2.2. Preparation of *M. citrifolia* extract

The different parts of *M. citrifolia* L. plant *viz*. fruit, leaf and seed were collected and dried at room temperature for 2-3 days and further dried at 60^0C. The dried leaf, fruit and seed were grounded to powder. About 100 g of the dried samples of leaf, fruit and seed bark were extracted with solvents *viz.* methanol, chloroform and acetone separately and incubated at room temperature for 48 h with stirring at regular interval. The extracts were filtered with the Whattman filter paper 41 and then the filtrate was vacuum dried by using rotary evaporator. The filtrate was stored in screw cap bottle at -20^0C for further use.

2.3.In-vitro antibacterial activity assay

The disc diffusion method was used to study the antimicrobial activity (Bauer *et al*, 1966).The Muellor Hinton agar (MHA) plate was inoculated with freshly grown bacterial culture of approximately 2.56 x 10^3 CFU/ml. About 50 µl of each plant extract (5 mg/disc) were loaded in the sterile filter paper disc (6 mm) and placed on the MHA plate. The pure solvents in equal volume served as negative control and gentamicin (10µg) and Chloramphenicol (10 µg) antibiotic disc was used as positive control. The plate was incubated at 37^0C for 18 - 24 h. After incubation the diameter of the zone of inhibition was measured by using HiMedia antibiotic scale.

Table (1) : The average percentage (%) of antibacterial activity of all plant extract

Extract	*Methanol*	*Chloroform*	*Acetone*	*Mean*
Leaf	56.8	52.9	60.5	56.7^{b}
Fruit	62	60.5	56	59.5^{b}
Seed	72.1	70.6	73.6	72.1^{a}
Mean	63.63	61.33	63.36	

Mean with different superscript varied significantly (p<0.05)

Table (2): Average zone of Inhibition (mm) of different parts of *M.ctirifolia* plants

	Leaf	*Seed*	*Fruit*	*Mean*
Methanol	10.0	12.52	4.48	9.0
Chloroform	9.72	11.92	4.4	8.68
Acetone	11.2	12.24	4.5	9.31
Mean	10.31^{a}	12.23^{a}	4.46^{b}	

Mean with different superscript varied significantly (p<0.05)

3. RESULTS AND DISCUSSION

The result of the antibacterial activity of the different parts of the *M.citirifolia* solvent extracts is given in Table 1. Among the different parts of the *M. citrifolia*, the seed extract produced best antibacterial activity (72.1%) followed by fruit extract (59.5%) and leaf extract (56.7%) respectively. The methanol was found to be the best solvent for extraction of antibacterial compounds from the *M. citrifolia* plants. The average antibacterial activity of methanol extract was 63.63 % followed by acetone extract 63.3% and chloroform extract (61.33%) respectively.

The zone of inhibition was found best in seed extract (12.23 mm) followed by leaf extract (10.31 m) and least with fruit extract (4.46 mm). Best zone of inhibition was produced by the acetone seed acetone extract (12.24 mm). Among the different solvents, the best activity was produced by methanol extract.

The average antibacterial activity of all the extracts is presented in Table 3. The methanol extract of leaf seed and fruit revealed that the maximum zone of inhibition was produced against *E.coli* (12.2mm) and the least was produced against *Klebsiella spp* (6.1 mm). The best activity was produced by the seed extract (12.52 mm) while the least was produced by fruit extract (4.4mm).

The chloroform extract revealed that the best activity was produced by seed extract 11.9 mm and the least activity was produced by fruit extract (4.4 mm). *E.coli* was the most susceptible organism which produced maximum zone of inhibition of 9.86 mm.

The acetone extract revealed that the best activity was produced by seed extract followed by leaf (11.2 mm) and fruit extract (4.5 mm) respectively. Among the different isolates, maximum activity was detected against *E. coli* (12.4 mm).

Overall, the antibacterial activity of the *M.citrifolia* revealed that the best antibacterial activity was produced by seed extract followed by leaf extract and fruit extract. All the bacterial pathogens were sensitive to all the extracts, however, *E.coli* and *Pseudomonas* spp. are found to be most sensitive to the leaf and seed extract.

Among the different accessions of the leaf extracts, the best antibacterial activity was produced by accession L3 (11.8 mm) followed by L1 (10.8mm), L2 (9.6mm), L5 (9.0 mm) and L4 (8.8mm) respectively. All the extracts produce better zone of inhibition against *Salmonella spp* (12.6mm) and the least zone of inhibition was obtained against *Klebsiella spp.* (6.2mm).

Table (3): Antibacterial activity of different extract of *M. citrifolia* (zone of inhibition in mm)

Extract		*E.coli*	*Pseudomonas spp.*	*Salmonella spp.*	*Staphylococcus aureus*	*Klebsiella spp*	*Mean*
Methanol	Leaf	10.8	8.8	12.6	11.6	6.2	10
	seed	12.8	12.4	12	13.4	12	12.52
	fruit	13	9.4	0	0	0	4.48
	Mean	***12.2***	***10.2***	***8.2***	***8.3***	***6.1***	
Chloroform	Leaf	8.8	8.4	11.6	11.0	8.8	9.7
	seed	11.6	12.6	11.2	13.0	11.2	11.9
	fruit	9.2	13	0.0	0.0	0.0	4.4
	Mean	***9.86***	***11.3***	***7.6***	***8.0***	***6.7***	
Acetone	Leaf	14.2	11	13.2	11.2	6.2	11.2
	seed	11.8	13.6	12.2	11.4	12.2	12.2
	fruit	11.2	11.2	0.0	0.0	0.0	4.5
	Mean	***12.4***	***11.9***	***8.5***	***7.5***	***6.1***	

The seed extracts of all the accessions showed antibacterial activity against all the tested organisms. Among the different seed extract the best inhibitory activity was produced by S1 (13.1 mm) followed by S3, S2, S5 and S4 respectively. The methanol extract (12.52±0.26 mm) showed better zone of inhibition compared to acetone (12.24±0.37 mm) and chloroform extracts (12.24±0.37 mm). All the seed extracts inhibited all the bacterial isolates and *Pseudomonas* spp showed maximum inhibition with all the seed extracts.

The best antibacterial activity was produced by F5 extract followed by F2, F1, F3 and F4 respectively. Chloroform extract (12.1±0.9 mm) showed the best zone of inhibition than the methanol (11.2±1.8 mm) and acetone extract (10.2±1.0 mm). All the fruit extracts produced zone of inhibition against *E.coli* and *Pseudomonas spp*. However, no inhibitory activity was produced against *Salmonella spp.*, *Staphylococcus aureus* and *Klebsiella spp*. The result of the fruit extract revealed that only fruit extracts F1 and F2 showed inhibitory activity and the same may be utilized against *E.coli* and *Pseudomonas* spp. infection.

The antibacterial activity of the different stages of fruit extract revealed that the best zone of inhibition was produced by matured stage of fruit (11.5 mm). The leaf extracts from all the accessions produced antibacterial activity against all the tested organisms. Among the various solvents used for extraction, the acetone extract (11.64±0.97 mm) produced best inhibitory zone of inhibition against all the bacterial isolates.

Overall analysis of the antibacterial activity of various extracts revealed that the best inhibitory activity was produced by the seed extract (12.23 mm) compared to the leaf and fruit extract. *E.coli* and the *Pseudomonas spp* were inhibited by all the extracts; however, *Salmonella spp.*, *Staphylococcus aureus* and *Klebsiella spp* were not inhibited by all the extracts. The analysis of antibacterial activity of all the accessions revealed that the best antibacterial activity was produced by accession 5 followed by accession 1, 2, 3 and 4 respectively. Seed extract of all accessions showed better inhibitory activity compared to the other plant extracts.

In the present study the activity of seed extract was found better than the leaf extract and fruit extract. The antibacterial activity of the *Morinda citrifolia* have also been reported (Usha *et al* 2010), wherein they have also reported the antibacterial activity of leaf extract of *M.citrifolia*. They have also reported the best antibacterial activity against *E.coli*. The antibacterial activity of *M.citrifolia* extracts was also reported earlier (Selvam et al, 2009). In the present study broad spectrum activity of *M.citiroflia* leaf, fruit and seed have been reported.

The antibacterial activity of the various extracts varied with the solvents used. Like in the present study the best result was obtained with methanol extract followed by acetone and chloroform extracts. The extraction of the antibacterial compounds depends on the polarity of the compounds. The variation in the antibacterial activity of the various solvents is due to the nature of the polarity of the solvents. Methanol is having higher polarity and thus they tend to dissolve different compounds from the plant materials dipped in them. Reports also revealed that ethanol and methanol are commonly used for extraction of antibacterial compounds (Karaman *et al*, 2003, Wei *et al*, 2008). Reports showed that methanol, ethyl acetate and hexane could be used for extraction of antibacterial and antifungal compounds from *M.citrifolia* L. fruit (Jayaraman *et al*, 2008) the present study, methanol and acetone extract produced best antibacterial activity. Since, methanol has high polarity it could dissolve both polar and non polar compounds and produced best activity.

The overall, result of the antibacterial activity from different accession of the *M.citrifolia* plants indicated that the best activity was obtained in accession number 5, which may be propagated further for extraction of antibacterial compounds from this plant.

All the parts of the plants could be used for extraction of antibacterial compounds. The study revealed that fruits should be harvested at the matured stage for extraction of better antibacterial compounds.

The use of *Morinda citrifolia* has been reported by several workers. The present findings revealed the antibacterial activity of the leaf, seed and fruit of various accessions of *M. citrifolia* plants. The identification of novel antimicrobial compounds from the different parts will lead to the development of therapeutics from this plant.

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