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Role of Aqueous, Methanol and Ethanol leaf Extract of *Morinda Citrifolia* (Indian Noni) in inhibiting on clinical *Pseudomonas aeruginosa* isolates

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Abstract
Morinda citrifolia is one of the most important traditional polynesian medicinal plant. This small evergreen tree is native of South Eastern Asia to Australia and now it has pantropical distribution all over the world. This plant has antifungal, antibacterial, anti-inflammatory and antiviral activities. The present study aqueous, methanol and ethanol extraction of leaf extract of Morinda citrifolia was carried out since this method is more suitable for clinical study. The leaf extract was prepared by conventional extraction method and it was assessed with minimum inhibitory concentration (MIC) method against clinical isolate of Pseudomonas aeruginosa. In the present study have found that ethanol extracts showed better antibacterial activity against P.aeruginosa when compared to methanol extract. Aqueous extract showed no activity against P.aeruginosa by MIC method. Solvent control also showed no activity against P.aeruginosa .The overall results indicates, methanol and ethanol extracts of M.citrifolia ,some useful drugs may be develop for the treatment of infectious diseases caused by P.aeruginosa.. The results revealed that plant extracts have inhibitory activity against the tested organism.

Keywords: *Morinda citrifolia, Antibacterial activity, Aqueous,Methanol, Ethanol leaf extract.*

Introduction

Pseudomonas aeruginosa is a non fermenting gram negative bacillus causing various infections such as, respiratory tract infections, blood stream infections, urinary tract infections and wound infections. Respiratory tract infections include pulmonary cystic fibrosis (CF) and is associated with a poor prognosis. These infections are difficult to treat due to its intrinsic and acquired resistance to various antibiotics and it is associated with a high mortality rate. *P. aeruginosa* is an important nosocomial pathogen.

Even though thousands of antimicrobial compounds exist, the ability of microbes to develop resistance to even the most powerful antimicrobial compounds is major drawback for treat the infections (Gerson *et al.*, 2006 & Conlon *et al.*, 2003). In order to keep pace with this ever increasing need for new antimicrobials, scientists are looking at herbal medicines to treat the infections.

Medicinal plants have immensely contributed to strength of human health since the time beyond the recorded history. Microorganisms cause a number of deleterious diseases in humans, animal and plants. 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. The roots, stem, bark, leaves, flowers and fruits of

the plants are involved in various combinations and almost 40 known and recorded herbal medicines are available. More than 160 neutraceutical compounds have been identified from the plant. *Morinda citrifolia* is one of such plant with wide medicinal use.

Morinda citrifolia (noni) belongs to the family Rubiaceae, also known as Indian mulberry, is indigenous to tropical countries and is considered as an important traditional folk medicine. *M. citrifolia* has a history of use in Polynesian traditional medicine for the treatments of infectious diseases. The indigenous tribes of Australia used the ripe fruits of *M. citrifolia* for treatment of respiratory infections. It has been reported to have a broad range of therapeutic effects including antibacterial, antiviral, antifungal, antitumor, anti inflammatory, anti-tubercular and antioxidant properties.

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. It has also been reported that leaves of *M. citrifolia* inhibited biofilm formation by *Streptococcus mutants* and *Streptococcus sanguinis*. However, scientific evidence of the benefits of the noni fruit juice is very limited but there are some anecdotal evidences for treatment of influenza.

Hence this study is aimed to determine the antimicrobial activity of various extracts of *Morinda citrifolia* leaves against *P.aeruginosa* by MIC (microbroth dilution method).

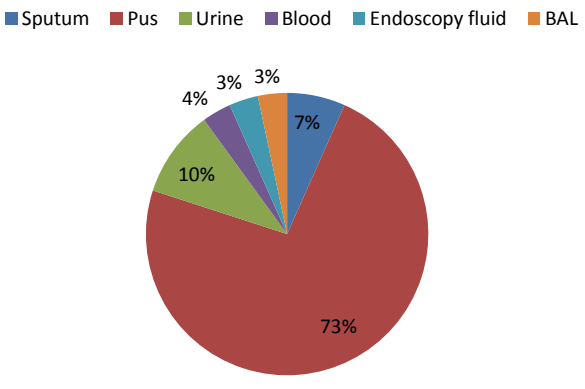
Materials and Methods

A total of 30 clinical *P.aeruginosa* isolates which were isolated from pus, urine, sputum, BAL, endoscopy fluid and blood were included in this study

Table.1 Details of the Isolates

S.NO	STRAIN ID	SOURCE
1	PAE143	PUS
2	PA44	PUS
3	PAE1O1	ENDOSCOPY FLUID
4	PA81	PUS
5	PA65	URINE
6	PA84	PUS
7	PA27	PUS
8	PA02	PUS
9	PA61	URINE
10	PA03	PUS
11	PAE140	SPUTUM
12	PAE158	SPUTUM
13	PAE124	BAL
14	PAE129	PUS
15	PAE09	PUS
16	PAE12	PUS
17	PA41	PUS
18	PAE148	BLOOD
19	PA23	PUS
20	PA04	PUS
21	PA70	PUS
22	PA46	PUS
23	PAE93	PUS
24	PA26	PUS
25	PAE151	PUS
26	PAE160	PUS
27	PAE172	PUS
28	PAE173	PUS
29	PA82	URINE
30	PA40	PUS

Figure 1: Source of P. aeruginosa isolates.



Plant Collection

Leaves of *Morinda citrifolia* were collected from the coconut gardens of Periyapalayam, Tamilnadu in the month of May 2015. The leaves were collected and washed with tap water, shade dried and then homogenized into fine powder and stored in air tight bottles.

Extract Preparation (Khuntia tapas kumar et al, 2010).

Aqueous Extraction

10 gram of dried plant material was mixed with 100 ml of aqueous solution. The extracts were filtered through the Whatman paper No.1 and syringe filter (0.45µm) and extracts kept at room temperature until the solvent gets evaporated completely. After drying was completed the above extracted materials were lyophilized for 24 hrs.

Ethanol Extraction

10 gram of dried plant material was mixed with 100 ml of ethanol solution. The extracts were filtered through the Whatman paper No.1 and syringe filter (0.45µm) and extracts kept at room temperature until the solvent gets evaporated completely. After drying the above extracted materials were lyophilized for 24 hrs.

Methanol Extraction

10 gram of dried plant material was mixed with 100 ml of Methanol solution. The extracts were filtered through the Whatman paper No.1 and syringe filter (0.45µm) and extracts kept at room temperature until the solvent gets evaporated completely. After drying the above extracted materials were lyophilized for 24 hrs.

Antimicrobial Activity

Micro broth dilution method was employed for testing antibacterial activity of *Morinda citrifolia* leaves extracts (Linday, 1962).

↓

Concentrations of extract ranging from 0.004mg/ml to 5mg/ml were prepared (Table.2).

↓

100µl of Muller Hinton broth was added to each well in a 96 well microtiter plate.

↓

100µl of extract was added to the 1st well and serially diluted.

↓

10µl of culture was added to all the wells.

↓

Incubated at 37˚C for 18-24 hours.

↓

Spot inoculation was done in MHA plates and incubated at 37˚C for 18-24 hours.

* Solvents without the extracts were used as control.

Table.2 Concentration of extracts

S.NO	Conc(Mg/ml)
1	5
2	2.5
3	1.25
4	0.625
5	0.312
6	0.156
7	0.0781
8	0.0390
9	0.019

Results

Aqueous Extract

Aqueous extract of *M.citrifolia* leaves showed no activity against all the tested *P. aeruginosa* isolates by MBC method. Figure 2 shows the varying concentrations of aqueous extract of *M.citrifolia* leaves ranging from 5mg/ml to 0.019mg/ml were marked as 1 to 9 and 10 as solvent control (water) and the picture depicts the MBC for 3 representative clinical isolates of *P.aeruginosa.*

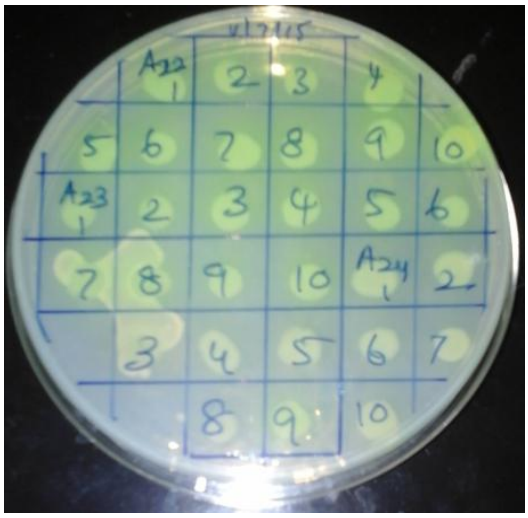


Fig.2 MBC test for *P.aeruginosa* by aqueous extract.

Ethanol Extract

All the 30 isolates were inhibited at the minimum concentration of 1.25mg/ml by ethanol extract of *M. citrifolia* leaves. Table 3 represents the MBC value of *M. citrifolia* by ethanol extract for all the 30 *P.aeruginosa* isolates. Figure 3 shows the varying concentrations of ethanol extract of *M.citrifolia* leaves ranging from 5mg/ml to 0.019mg/ml were marked as 1 to 9 and 10 as solvent control (water) and the picture depicts the MBC for 3 representative clinical isolates of *P.aeruginosa.*

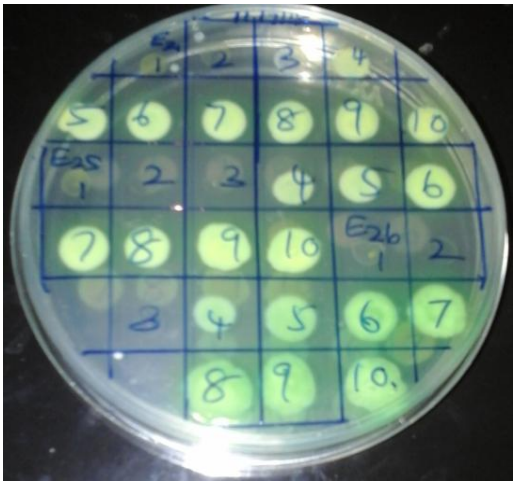


Fig.3 MBC test for *P. aeruginosa* by Ethanol extract.

Table.3 MBC results for *P.aeruginosa* by ethanol extract

ETHANOL EXTRCT	5mg/ml	2.5mg/ml	1.25mg/ml	0.625mg/ml	0.312mg/ml	0.156mg/ml	0.0781mg/ml	0.039mg/ml	0.019mg/ml
PAE143	-	-	-	+	+	+	+	+	+
PA44	-	-	-	+	+	+	+	+	+
PAE1O1	-	-	-	+	+	+	+	+	+
PA81	-	-	-	+	+	+	+	+	+
PA65	-	-	-	+	+	+	+	+	+
PA84	-	-	-	+	+	+	+	+	+
PA27	-	-	-	+	+	+	+	+	+
PA02	-	-	-	+	+	+	+	+	+
PA61	-	-	-	+	+	+	+	+	+
PA03	-	-	-	+	+	+	+	+	+
PAE140	-	-	-	+	+	+	+	+	+
PAE158	-	-	-	+	+	+	+	+	+
PAE124	-	-	-	+	+	+	+	+	+
PAE129	-	-	-	+	+	+	+	+	+
PAE09	-	-	-	+	+	+	+	+	+
PAE12	-	-	-	+	+	+	+	+	+
PA41	-	-	-	+	+	+	+	+	+
PAE148	-	-	-	+	+	+	+	+	+
PA23	-	-	-	+	+	+	+	+	+
PA04	-	-	-	+	+	+	+	+	+
PA70	-	-	-	+	+	+	+	+	+
PA46	-	-	-	+	+	+	+	+	+
PAE93	-	-	-	+	+	+	+	+	+
PA26	-	-	-	+	+	+	+	+	+
PAE151	-	-	-	+	+	+	+	+	+
PAE160	-	-	-	+	+	+	+	+	+
PAE172	-	-	-	+	+	+	+	+	+
PAE173	-	-	-	+	+	+	+	+	+
PA82	-	-	-	+	+	+	+	+	+
PA40	-	-	-	+	+	+	+	+	+

Methanol Extract

Among 30 isolates, 27 isolates were inhibited at the concentration of 2.5mg/ml and remaining 3 isolates were inhibited at the concentration of 5mg/ml by methanol extract of *M. citrifolia* leaves. Table 3 represents the MBC value of *M. citrifolia* by methanol extract for all the 30 *P.aeruginosa* isolates. Figure 4 shows the varying concentrations of methanol extract of *M.citrifolia* leaves ranging from 0.019mg/ml to 5mg/ml were marked as 1 to 9 and 10 as solvent control (water) and the picture depicts the MBC for 3 representative clinical isolates of *P.aeruginosa.*

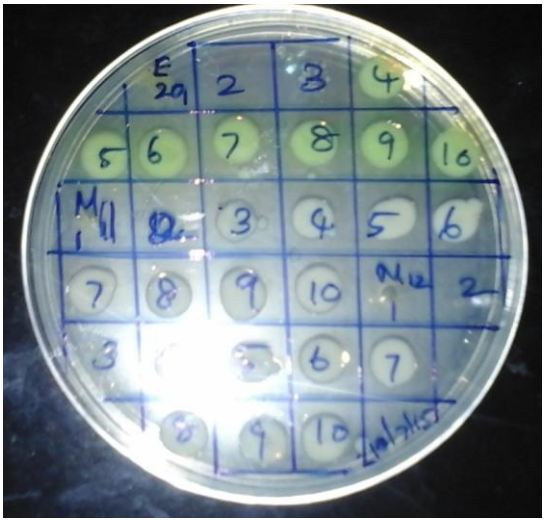


Fig.4 MBC test for *P. aeruginosa* by Methanol extract.

Table.4: MBC results for *P.aeruginosa* by Methanol extracts.

METHANOL EXTRCT	5mg/ml	2.5mg/ml	1.25mg/ml	0.625mg/ml	0.312mg/ml	0.156mg/ml	0.0781mg/ml	0.039mg/ml	0.019mg/ml
PAE143	-	-	+	+	+	+	+	+	+
PA44	-	-	+	+	+	+	+	+	+
PAE1O1	-	-	+	+	+	+	+	+	+
PA81	-	-	+	+	+	+	+	+	+
PA65	-	-	+	+	+	+	+	+	+
PA84	-	-	+	+	+	+	+	+	+
PA27	-	-	+	+	+	+	+	+	+
PA02	-	-	+	+	+	+	+	+	+
PA61	-	-	+	+	+	+	+	+	+
PA03	-	-	+	+	+	+	+	+	+
PAE140	-	-	+	+	+	+	+	+	+
PAE158	-	-	+	+	+	+	+	+	+
PAE124	-	-	+	+	+	+	+	+	+
PAE129	-	-	+	+	+	+	+	+	+
PAE09	-	-	+	+	+	+	+	+	+
PAE12	-	-	+	+	+	+	+	+	+
PA41	-	-	+	+	+	+	+	+	+
PAE148	-	-	+	+	+	+	+	+	+
PA23	-	-	+	+	+	+	+	+	+
PA04	-	-	+	+	+	+	+	+	+
PA70	-	+	+	+	+	+	+	+	+
PA46	-	+	+	+	+	+	+	+	+
PAE93	-	+	+	+	+	+	+	+	+
PA26	-	-	+	+	+	+	+	+	+
PAE151	-	-	+	+	+	+	+	+	+
PAE160	-	-	+	+	+	+	+	+	+
PAE172	-	-	+	+	+	+	+	+	+
PAE173	-	-	+	+	+	+	+	+	+
PA82	-	-	+	+	+	+	+	+	+
PA40	-	-	+	+	+	+	+	+	+

Discussion

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* leaves were screened for antibacterial effect against the *P.aeruginosa*. Minimum inhibitory concentration (MIC) values favorably ensure that *M. citrifolia* could be recommended to treat infectious disease.

The yield of *M. citrifolia* leaves extracts was higher in ethanol, compared to methanol and aqueous. The petroleum ether and alcoholic extract of *Morinda citrifolia* leaves were screened for antimicrobial properties against *E. coli, B.subtilis* and *S. aureus.* 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. Minimum inhibitory concentration against *P. seruginosa* showed maximum MIC at 1.25g/ml. Minimum inhibitory concentration ranges from 0.009mg/ml to 5mg/ml. Growth of tested bacteria was not found in the subculture of the microtiter wells above the minimum inhibitory concentration (MIC) in the tested leaf extract. Similar results were observed by Esath who reported the inhibitory effect of *Morinda citrifolia* against the growth of *E.coli, P.aeruginosa, S.aureus, Streptococcus sp., K.pneumoniaee, Sh. flexneri, P. mirabilis, P.diminuta, P.fluorescens* and *E.cloacae* (Esath *et al.*, 2012). MIC values tend to vary against organism based on the solvent chosen evaluated *B. 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. Our study was in par with the results obtained. 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.

The increased frequency of resistance to commonly used antibiotics led to search for newer, effective, cheap and easily affordable drugs in the management of infectious diseases. The methanol extracts inhibited most of the bacterial isolates significantly compared to ethanol and ethyl acetate extract. Many studies revealed that the methanol extracts of plants inhibit the growth of bacteria more than aqueous extracts of plants and found to possess more inhibitory effect than other extracts. In this study, our results showed that ethanol extracts of *M. citrifolia* leaves gave favourable results against all the 30 bacterial isolates tested than methanol and aqeous extracts. This trend to show that the active ingredients of plant parts are better extracted with ethanol than other solvents. Generally, plant extracts are usually more active against gram positive bacteria than gram negative bacteria (Basri and Fan, 2005). This may be due to the permeability barrier provided by the cellwall or to the membrane accumulator mechanisms.

M. citrifolia has been classified as a medicinal herb due to its therapeutic properties. Various parts of the plant, including roots, stems, leaves and fruit have been consumed solely on the basis of the assumption that it possesses healing properties. In the present study, *M.citrifolia* leaves antibacterial property has been screened and the results showed effect on the growth of most of the isolates and MIC values auspiciously determines that plant parts of *M.citrifolia* can be used in the treatment of infectious disease. Methanolic extracts inhibited most the tested microorganisms than ethanol and ethyl acetate extracts by Abd Aziz *et al.*, 2011). Acceptance of medicines from plant origin as an alternative form of healthcare is increasing because they are serving as promising sources of novel antibiotic prototypes (Kodura *et*

al., 2006). Some of the phytochemical compounds e.g. glycosides, saponin, tannin, flavonoids, trepenoid, alkaloid have variously been reported to have anti-microbial activity. The complications in the antimicrobial activity of propolis could be due to differences in its chemical components. It has also been reported that the samples collected from different geographic origin with different climates and vegetations show different antibacterial activities (Kashi *et al.*, 2011).

The present study shows that the methanol, ethanol and ethyl acetate extracts have inhibitory activity against the *P. aerugenosa*. The inhibition of the growth of these organisms *in vitro* by the extracts may be due to the presence of some active constituents in the extracts. These active principles may have acted alone or in combination to inhibit the growth of the bacterial organisms. The medicinal uses of these plants to heal diseases including infectious one has been extensively applied by people. The problem of microbial resistance is growing and the outlook for use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to develop research to better understand the genetic mechanisms of resistance and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient.

The overall results indicate promising baseline information for the potential uses of solvent extracts of *M.citrifolia* leaf in the treatment of infectious disease. The most active extracts can be subjected to isolation of the therapeutic antimicr-obials and undergo further pharmacological evaluation. This observation is in agreement with who reported that ethyl acetate extracts of *M. citrifolia* fruits showed significant activity against *T.mentagrophytes* and *S.aureus* when compared to other tested organism**.** But in this study, the maximum activity was recorded for ethanol extract against *P.aerugenosa* (1.25mg/ml). Arpornsuwan and Punjanon (2006) reported that the methanolic extract of *M. citrifolia* fruit was much more effective on breast cancer cells and neuro blastoma 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 promoter (unpublished data).In this study ethanol extract shows the better activity compared to methanol activity. The overall results indicate promising baseline information for the potential uses of the ethanol and methanol extracts of *M. citrifolia* leaves, it may use in the treatment of infectious diseases.

Summary and Conclusion

The ethanol extracts showed better antibacterial activity against *P.aeruginosa* when compared to methanol extract. Aqueous extract showed no activity against *P.aeruginosa* by MIC method. Solvent control also showed no activity against *P.aeruginosa* .The overall results indicates, methanol and ethanol extracts of *M.citrifolia* ,some useful drugs may be develop for the treatment of infectious diseases caused by *P.aeruginosa.*

Acknowledgment

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Competing Interests

No competing interest declared.

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