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Effect of *Morinda citrifolia* extracts on *in-vitro* growth of *Ralstonia solanacearum*

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ABSTRACT

Bacterial wilt is a major disease in solanaceaous plants caused by a bacteria called Ralstonia solanacearum. It affects large verities of solanaceaous plants worldwide. With an aim to develop effective antibacterial agent without any residual affect, the present study was conducted to analyze the in-vitro antibacterial potential of Morinda citrifolia against two different isolates of Ralstonia solanacearum. The Morinda citrifolia leaf and fruits were collected, dried and extracted using acetone and chloroform. The antibacterial activity of the extracts was assayed by using disc diffusion method. The results revealed that the average zone of inhibition of the leaf extracts was more in the chloroform extract (24mm) than in the acetone extract (23.5mm). However, no significant difference was observed with both the extracts. The fruit acetone extract produced 16.5mm zone of inhibition while the fruit chloroform extract produced 16mm zone of inhibition. The overall inhibitory activity of the leaf and fruit extracts against Ralstonia solanacearum (RSN6) isolate showed that 58.82% of the antibiotics produced less inhibitory activity than the fruit and leaf extracts. The inhibitory activity of leaf and fruit extracts against Ralstonia solanacearum (RSN12) isolate showed that 70.59% of antibiotics produced less inhibitory activity than the fruit and leaf extracts of Morinda citrifolia. The results revealed that both the extracts have produced inhibitory activity against Ralstonia solanacearum and the same could be used to extract antibacterial compounds. The potential of Morinda citrifolia extracts was found much better than most of the antibiotics.

Key words: Antibacterial activity, *Morinda citrifolia, Ralstonia solanacearum.*

INTRODUCTION

Bacterial wilt, is a major problem in the tomato, brinjal however, it affects wide range of important crop plants including potato, tobacco, tomato, groundnut, banana etc [1]. The disease is caused by a Gram –ve bacteria; *Ralstonia solanaceaurm*. In A&N Islands bacterial wilt is known to be endemic all over the island, including forest soils. The pathogen survives in soil for

extended periods without a host plant. It enters roots through wounds, which may be caused by insects, nematodes, and cultivation. High temperature and high soil moisture favor disease development. Control of these diseases requires integrated cultural practices and chemical sprays with copper compounds, but available measures are not effective and one of the major limitations of using chemical control agents is the development of resistance in bacteria [2]. Use of agrochemicals is becoming less favorable because of environmental pollution and detrimental effect on a variety of non–target organisms [3]. Biological methods of control including use of natural plant products have therefore been preferred because most of them are locally available, environmental friendly, have no side effect and development of resistance is rare [4].

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. The research on the use of different solvent extracts of *M.citrifolia* revealed broad spectrum antibacterial and antifungal activity [5,6,7].

The use of plant extracts is found to be an effective way of controlling plant diseases compared to synthetic chemicals as plant extracts have several advantages over it. Therefore, there is an urgent need to search for effective, safe and biodegradable alternative pesticides. Very little work has been done to investigate the use of natural plant products to control bacterial wilt. The study was therefore carried out to determine the effect of *Morinda citrifolia* plant extracts on the *in-vitro* growth and development of *R. solanacearum* colonies.

MATERIALS AND METHODS

Collection and identification of plant materials

Fresh and disease free leaf and fruits of *M. citrifolia* were collected from Horticultural Research farm complex, CARI. Plant materials were washed thoroughly with tap water and once with sterile distilled water and then dried under shade.

Test Bacteria

Bacteria namely *Ralstonia solanacearum* was collected from the Plant Pathology Section of the CARI. The isolates were isolated from the infected brinjal plant and identified based on their physiological and biochemical characteristics. The bacteria were maintained on nutrient agar slant at 4^0C and sub cultured periodically on fresh medium.

Antibiotic Sensitivity Test

The antibiotic sensitivity test was done as per the disc diffusion method [8]. Briefly, the overnight culture of *Ralstonia solanacearum* was plated on to the Muellor Hinton Agar. The standard antibiotics disc (octadisc, Himedia) were used to study the antibiotic sensitivity pattern of the isolates. The plate was incubated at 37^0C for 24 h and the zone of inhibition was measured with the Himedia antibiotic scale.

Preparation of *M. citrifolia* solvent extracts

The different parts of *M.citrifolia* L. plant *viz*. fruit and leaf were collected and dried at room temperature for 2-3 days and further dried at 60^0C. The dried leaf and fruit were grounded to powder. The dried leaf and fruit samples were extracted with solvents *viz.* chloroform and acetone separately and incubated at room temperature for 48 h with stirring at regular interval. The extracts were filtered with the Whatman 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 antibacterial activity assay.

***In-vitro* antimicrobial activity assay**

The disc diffusion method was used to study the antimicrobial activity. The Muellor Hinton agar (MHA) plate was inoculated with freshly grown bacterial culture of approximately 4.56 x 10^3 CFU/ml. About 20 µl of each plant extract 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 chloramphenicol antibiotic disc (30 µg) 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.

The data were statistically analyzed as a randomized complete block design by SAS (1990) software (SAS Inst. Inc., Cary, NC).

RESULTS AND DISCUSSION

Table 1. Antibiotic sensitivity pattern of *Ralstonia solanacearum* isolates

S. No.	Antibiotics	Concentration (µg)	Zone of inhibition (mm)	
			RSN6	RSN12
1	Ampicillin	25	0	0
2	Cephalothin	10	0	0
3	Chloramphenicol	25	17	19
4	Ciprofloxacin	10	33	29
5	Clindamycin	25	0	0
6	Colistin	10	0	0
7	Co-trimazole	25	25	18
8	Erythromycin	15	23	22
9	Gentamycin	30	24	22
10	Gentamycin	10	21	19
11	Nitrofurantonin	300	10	0
12	Ofloxacin	25	30	32
13	Oxacillin	5	0	0
14	Penicillin-G	10	0	11
15	Streptomycin	10	12	12
16	Tetracyclin	25	27	30
17	Vancomycin	10	0	0
	Mean ± S.E.		**13.06±3.04^{NS}**	**12.59±2.93^{NS}**

Means with same letter are not differ significantly ($p<0.05$)

Figure 1. Zone of inhibition of RSN6 and RSN 12 isolates against different antibiotics

Antibacterial activity of leaf extract

The solvent extraction of fruit and leaf of *M.citrifolia* shows that the percentage of recovery was more in leaf chloroform (7.82 %) followed by fruit acetone (7.5%), leaf acetone (7.3%) and fruit chloroform (3.8%) respectively. The chloroform solvent produced more antibacterial compounds compared to the acetone solvent.

Antibiotic sensitivity profile of *Ralstonia solanacearum*

The results of the antibiotic sensitivity test revealed that 41.17% of the antibiotics did not show zone of inhibition with RSN 6 and RSN 12 isolates (Table 1). The average zone of inhibition of 13.06±3.04 mm and 12.59±2.93 mm were produced against RSN 6 and RSN 12 isolates respectively (Figure 1) The maximum zone of inhibition of 33 mm was produced against both the isolate. Almost similar type of inhibitory activity was obtained with respect to 17 standard broad spectrum antibiotics. The best zone of inhibition was obtained with ciprofloxacin (33 mm).

The leaf acetone extract produced 24±0.44 mm zone of inhibition against RSN 6 and 23±0.25 mm against RSN 12 isolate (Table 2). An average of 23.5±0.31 mm zone of inhibition was obtained against both the Ralstonia which is much better than most of the antibiotics. The leaf chloroform extracts produced 26.0±0.25 and 22.3±0.26 mm zone of inhibition against RSN6 and RSN 12 isolate. The average zone of inhibition was more in the chloroform extracts than the acetone extracts; however, no significant difference was obtained with both the extracts. Both the extracts showed more activity against the RSN 6 isolate compared to the RSN 12 isolate.

Antibacterial activity of fruit extracts

The fruit acetone extract produced 14.3±0.26 mm zone of inhibition against RSN 6 and 19.6±0.3 mm against RSN 12 isolate (Table 2). An average of 16.5±1.58 mm zone of inhibition was obtained against both the Ralstonia. The fruit chloroform extracts produced 15.0±0.25 and 17.33±0.26 mm zone of inhibition against RSN 6 and RSN 12 isolate. The average zone of inhibition was more in the acetone extract than the chloroform extract. However, no significant difference was observed with both the extracts. Both the extracts showed more activity against the RSN 12 isolate compared to the RSN 6 isolate.

The antibacterial activity of the solvent extracts revealed that the best activity was observed with leaf extract which produced 46% more activity than the fruit extract. Among the solvents the

chloroform extract produced 7.69% more activity over the acetone extract. Both the isolates were sensitive to the antibacterial compounds of fruit and leaf extract, however, the RSN6 isolate was more resistant than the RSN 12 isolate.

Table 2: antibacterial activity of solvent extract

	RSN 6	RSN12	Mean ± S.E.
Leaf acetone	24±0.44	23±0.25	**23.5±0.31**^{a}
Leaf Chloroform	26.0±0.25	22.3±0.26	**24.0±1.26**^{a}
Fruit acetone	14.3±0.26	19.6±0.3	**16.5±1.58**^{b}
Fruit chloroform	15.0±0.25	17.33±0.26	**16.0±0.63**^{b}
Mean ± S.E.	**19.7±2.74**^{NS}	**20.25±1.23**^{NS}	

Means with same letter are not differ significantly (p<0.05)

Comparison of antibacterial activity of solvent extracts with Antibiotics

The antibacterial activity of the leaf and fruit extracts of *Morinda citrifolia* was 58.82% more than the antibiotics against the RSN 6 isolate of *Ralstonia solanacearum* (Table 3). Only 7 antibiotics produced more inhibitory activity than the morinda fruit and leaf extracts. The best inhibitory activity was produced in descending order by leaf chloroform (26 mm), leaf acetone (24 mm), fruit chloroform (15 mm) and fruit acetone (14mm) respectively against RSN 6 isolate. The difference of average inhibitory activity against the standard antibiotics was more in leaf chloroform (12.94±3.05 mm), followed by leaf acetone (6.82±2.74 mm), fruit chloroform (1.94±3.05 mm) and fruit acetone (0.94±3.05 mm) against RSN 6 isolate.

Table 3: Difference of zone of inhibition between solvent extracts and antibiotics

Antibiotics	RSN 6				
	Leaf acetone	Fruit acetone	Fruit Chloroform	Leaf chloroform	Mean±S.E
Ampicillin	24	14	15	26	**19.75**±3.07^{a}
Cephalothin	24	14	15	26	**19.75**±3.07^{a}
Chloramphenicol	7	-3	-2	9	**2.75**±3.07^{bcde}
Ciprofloxacin	-9	-19	-18	-7	**-13.25**±3.06^{g}
Clindamycin	24	14	15	26	**19.75**±3.06^{a}
Colistin	24	14	15	26	**19.75**±3.07^{a}
Co-trimazole	-1	-11	-10	1	**-5.25**±3.07^{efg}
Erythromycin	2	-9	-8	3	**-3**±3.19^{defg}
Gentamycin	0	-10	-9	2	**-4.25**±3.07^{efg}
Gentamycin	3	-7	-6	5	**-1.25**±3.07^{cdef}
Nitrofurantonin	14	4	5	16	**9.75**±3.07^{abc}
Ofloxacin	-6	-16	-15	-4	**-10.25**±3.07^{fg}
Oxacillin	0	14	15	26	**13.75**±5.33^{ab}
Penicillin-G	0	14	15	26	**13.75**±5.33^{ab}
Streptomycin	12	2	3	14	**7.75**±3.065^{bcd}
Tetracyclin	-2	-13	-12	-1	**-7**±3.19^{efg}
Vancomycin	0	14	15	26	**13.75**±5.33^{ab}
Mean ± S.E.	**6.82±2.74**^{ab}	**0.94±3.05**^{b}	**1.94±3.05**^{b}	**12.94±3.05**^{a}	
% Inhibition more than the extract	**76.47**	**52.94**	**52.94**	**82.35**	

Means with same letter are not differ significantly (p<0.05)

The inhibitory activity of the fruit and leaf extracts against RSN 12 showed that 70.59 % of antibiotics showed less inhibitory activity than the fruit and leaf extracts of the *Morinda citrifolia* (Table 4). Only 5 antibiotics produced more inhibitory activity than the fruit and leaf extracts. The difference of inhibitory activity against the standard antibiotics was more in leaf acetone (10.41±2.94 mm) followed by leaf chloroform (9.41±2.94 mm) fruit acetone (6.41±2.94 mm) and fruit chloroform (4.41±2.94 mm) against RSN 12 isolate.

Table 4. Difference of zone of inhibition between solvent extracts and antibiotics

Antibiotics	**RSN 12**				
	Leaf acetone	**Fruit acetone**	**Fruit Chloroform**	**Leaf chloroform**	**Mean** ±S.E
Ampicillin	23	19	17	22	20.25±1.38^{a}
Cephalothin	23	19	17	22	20.25±1.38^{a}
Chloramphenicol	4	0	-2	3	1.25±1.38^{c}
Ciprofloxacin	-6	-10	-12	-7	-8.75±1.38^{d}
Clindamycin	23	19	17	22	20.25±1.38^{a}
Colistin	23	19	17	22	20.25±1.38^{a}
Co-trimazole	5	1	-1	4	2.25±1.38^{c}
Erythromycin	1	-3	-5	0	-1.75±1.38^{c}
Gentamycin	1	-3	-5	0	-1.75±1.38^{c}
Gentamycin	4	0	-2	3	1.25±1.38^{c}
Nitrofurantonin	23	19	17	22	20.25±1.38^{a}
Ofloxacin	-9	-13	-15	-10	-11.75±1.38^{d}
Oxacillin	23	19	17	22	20.25±1.38^{a}
Penicillin-G	12	8	6	11	9.25±1.38^{b}
Streptomycin	11	7	5	10	8.25±1.38^{b}
Tetracyclin	-7	-11	-13	-8	-9.75±1.38^{d}
Vancomycin	23	19	17	22	20.25±1.38^{a}
Average	**10.41±2.94^{NS}**	**6.41±2.94^{NS}**	**4.41±2.94^{NS}**	**9.41±2.94^{NS}**	
% Inhibition more than the extract	**82.35**	**70.59**	**52.94**	**82.35**	

Means with same letter are not differ significantly ($p<0.05$)

Overall, the best inhibitory activity was produced by leaf chloroform and the least activity was produced by fruit chloroform. The inhibitory activity was more in RSN 12 compared to RSN 6 (Figure 2). The result revealed that both the extracts have produced inhibitory activity against the *Ralstonia spp* and the same could be used to extract antibacterial activity against the *Ralstonia spp*. The potential of the *Morinda citrifolia* extracts was found much better than most of the antibiotics and the same may be useful against the *Ralstonia solanacearum.*

In the present study the leaf chloroform extract produced better antibacterial activity than the fruit extract. It was also observed that the effect was higher in chloroform extract and less in acetone extract. This indicates that the active constituents of the plant parts have more ability to dissolve in chloroform solvent than the acetone solvent used in this study. The percent yield of antibacterial compounds also revealed that the yield was higher in leaf chloroform extract compared to the other extracts. The antibacterial activity of *M.citrifolia* was also reported by some workers [5,6,7], however, the antibacterial nature of this plant against the *Ralstonia solanacearum* is not reported earlier.

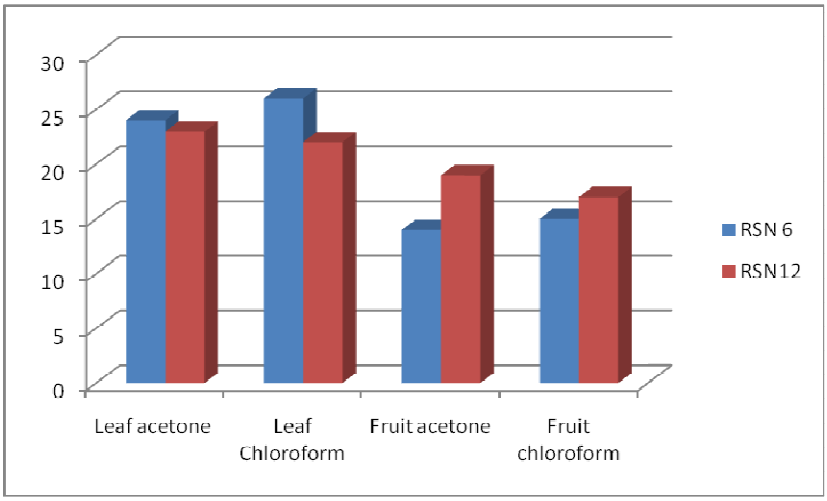

Figure 2. Antibacterial activity against RSN 6 & RSN 12

Antibacterial properties of plant extracts against human pathogenic bacteria have been reported by several studies but only a few studies have been done on plant pathogens using plant extracts. This study demonstrated that compounds extracted from the *Morinda citrifolia* fruit and leaves using two organic solvents vary in their efficiency in inhibiting bacterial growth. The difference observed in the antibacterial activity of the extracts is likely to be due to the solubility of active compounds in the chloroform and acetone extract. The two solvents have different polar ability. Chloroform is an effective solvent for alkaloids in their base form and thus plant material is commonly extracted with chloroform for pharmaceutical processing.

Synthetic pesticides are nowadays widely used for the control of plant diseases throughout the world because of their higher effectiveness in controlling disease causing organisms. However, excessive and unsystematic application of these chemicals has created several environmental and health hazards and also some phytopathogens have been developed resistance [9].Green plants have been shown to represents a reservoir of effective chematherapeutants and can provide valuable sources of natural pesticides [10]. The use of *M.citrifolia* as a therapeutic agent for control of *Ralstonia solannacearum* will be of great use as the disease is highly prevalent and the biological control of the wilt will certainly enhance the quality of the plant products. Green plants are found to be an effective reservoir for the bioactive molecules and can provide valuable sources for the discovery of natural pesticides [4]. Therefore, in recent years medicinal plant extracts are intensively analyzed with an aim of isolating novel bioactive compounds.

The antibacterial activity of the *M.citrifolia* showed higher activity than some of the standard antibiotics which can be effectively used against the resistant antibiotics. The indiscriminate usage of antibiotics will produce antibiotic resistance and residue in the end products; however with the use of plant extracts it can be avoided.

The antibacterial effect of crude medicinal plant extract of *Ocimum gratisimum, Brassica oleracae* and *Ipomoea batatas* on *Ralstonia solanacearum* were also reported [11]. In the present study similar type of activity were reported with the use of *M. citrrifolia* plants. The active

principles present in the plants are influenced by many factors such as age of plant, part of the plants, solvents etc.

The antibacterial activity of Ralstonia with plant extracts have been reported earlier [12,13,14,15]. However, the antibacterial activity of *Morinda citrifolia* extract against *Ralstonia solanacearum* is never reported earlier. The finding of the results is encouraging and could be used as a source of antimicrobial compounds for the control of bacterial wilt caused by *Ralstonia solancearum.*
It was evident that the use of *Morinda citrifolia* solvent extracts has a potential to substitute the antibiotics to control the infection. This kind of biological approach would be economical, safe, environmental friendly. These plants are also available in plenty and farerms can use it for control of wilt in the solanaceus crops. However, the chemical compounds are yet to be isolated from this plants which requires further detail study.

CONCLUSION

This study revealed the antibacterial activity of different solvent extracts of leaf and fruit of *Morinda citrifolia* against *Ralstonia solanceasrum*, the causal agent of bacterial wilt in plants. The c leaf chloroform extract was found better than the acetone extracts. The antibacterial activity of leaf and fruit are found much better than some of the broad spectrum antibiotics. This is the first report of *Morinda citrifolia* on the *in-vitro* antibacterial activity of *Ralstonia solancearum*. The results are very encouraging and the identification of the novel antibacterial compounds could be useful in the control of bacterial wilt infection in plant caused by *Rasltonia solanacearum.*

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