Antibacterial activity of water extracts of different parts of *Morinda citrifolia* grown in Sri Lanka

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Abstract- This study examined the antibacterial potential of water extracts of different parts of *Morinda citrifolia* (Family: Rubiacae) plants which is used/indicated in Sri Lankan traditional and folk medicine to wash old cutaneous wounds, cuts, abrasion, rashes, bruises or burns, and as a throat gargle. This was tested in triplicate, *in vitro*, using agar disc diffusion bio assay against Gram positive human pathogenic bacteria, *Staphylococcus aureus* (ATCC 25923) and Gram negative bacteria , *Escherichia coli* (ATCC 35218). Concentration of water extracts tested were 500,750 and 1000 µg/disk. Gentamicin (10 µg/disk) was used as the positive control. The results showed that none of the extracts (roots, fruits, flower, stem bark or combination of these parts) was effective against *E. coli* whilst fruit (diameter of inhibition zone 2.93 ±0.03mm), stem bark (5.1 ±0.03mm) and combined extract (9.46 ± 0.03mm) exhibited antibacterial activity against *S. aureus.* The reference drug, Gentamicine induced an inhibition zone of 13.98 ±0.04mm against *S. aureus* and 15.02 ±0.03 mm against *E. coli*. It is conducted that water extracts of different parts of *M. citrifolia* plant has mild to moderate antibacterial activity against *S. aureus*, a commonly encountered pathogen in cutaneous wounds. In addition, the results justify its use in Sri Lankan ethnomedicine as a topical cleaning agent for infected skin wounds.

Index Terms- Morinda citrifolia, antibacterial activity, water extract, traditional medicine, folk medicine Sri Lanka

I. Introduction

Morinda citrifolia (Family: Rubiaceae) is a small evergreen tree, with straight stem and a long elliptical shaped dark green and shining leaves. Its flowers are white and tubular, and the fruits are ovoid and yellow. The seeds are triangular in shape and reddish brown in color, and have an air sac attached to one end. The plant has several vernacular names such as noni, Indian mulberry, polynesia fruit, bambo nonu, cheese fruit, nono or ahu. The plant can grow in infertile, acidic and alkaline soils in extremely dry and wet areas throughout the tropics ranging from Brazil to Australia [1, 2, 3]

In folk and traditional medicines of various countries, extracts of different parts of this plant are claimed to be used for centuries, to cure and prevent variety of illnesses and health related conditions. These include arthritis, atherosclerosis, diabetes, gastric ulcers, gingivitis, hypertension, colds and influenza, cancer, eye problems, menstrual disorders, inflammation, heart diseases, various infections [1, 2, 3]. Interestingly, some of these traditional claims are experimentally proven [1, 2].

In Sri Lankan traditional and folk medicine water extracts of various part of this plant is used in cleaning/washing cutaneous wounds, cuts, abrasion, rashes, bruises, burns, and also as a throat gargle [4, 5]. However, the validity of this use is not scientifically validated or refuted. Therefore this study was undertaken to test antibacterial activity of water extracts of various parts of this plant *in vitro* using agar disc diffusion bioassay against Gram positive bacteria, *Staphylococcus aureus* and Gram negative bacteria, *Escherichia coli*. These two bacteria species are commonly encountered as human pathogens in cutaneous infections [6].

II. Materials & Methods

Collection & Authentication:

Whole matured plants with flowers and fruits were collected from Mallawapitiya area in Kurungegala district, in Sri Lanka (GPS 7°28'21.7"N 80°23'23.8"E), in December 2015. The whole plant had been identified and authenticated by a botanist at the National Herbarium, Peradeniya, Sri Lanka.

A voucher specimen of stem bark of *M. citrifolia* (MC/ 02/2015), seeds of *M. citrifolia* (MC/ 03/2015), roots of *M. citrifolia* (MC/ 04/2015), flowers of *M. citrifolia* (MC/05/2015) and leaves (MC/06 / 2015) of *M. citrifolia* were deposited at the Department of Medical Laboratory Sciences, General Sir John Kotelawala Defence University, Sri Lanka.

Preparation of aqueous extracts of M. citrifolia

The roots, seeds, flowers, leaves and stem bark were removed from the plants and were air dried in shade for 2-4 days and were cut in to small pieces separately. In order to prepare

aqueous extracts for each sample separately, twenty three grams (23 g) from each sample was boiled slowly in 92 ml of distilled water for approximately for 3 hours until the volume was reduced to 18 ml. The prepared aqueous sample was left for cooling down and stored securely at 4°C for later use.

There was another separate aqueous sample prepared by adding 23 g of roots, 05 g of seeds, 05 g of flower, 23g of leaf and 23 g of stem bark into 316 ml of distilled water. This sample was boiled for about 5 hours until the final volume of the solution reached 62 ml. This sample was labeled as the combined sample and store at 4°C for later use.

Antibacterial Activity Screening

Prepared aqueous samples (labelled as roots, seeds, flowers, leaves, stem bark and combined) were evaluated for antibacterial activity using the disc diffusion method as described by clinical and laboratory institute [7] Distilled water was used as the negative control while Gentamicin (10 µg/disk) was used as the positive control. The growth medium used for this experiment was Muller Hinton Agar and the antibacterial activities were determined against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 35218) at following concentrations: 500 µg of plant extract /disk,750 µg of plant extract /disk and 1000 µg of plant extract /disk. The antibacterial activity was examined, in triplicate, for each sample and the diameter of the inhibition zone (in mm) for each extract against the above mentioned strains were measured and recorded.

Statistical Analysis:

The data was statistically analyzed by using Non Parametric-Kruskal-Wallis Test in SPSS (17th Version) software package. The data are expressed as mean inhibition zone diameter ± Standard Error of the Mean (SEM) and Pearson's regression analysis was performed to evaluate dose-dependencies. Significance level was set at $p < 0.05$.

III. Results

The results are summarized in Table 1. As shown, none of the extracts exereted any antibacterial activity (in term of the diameter of zone of inhibition) against Gram negative bacteria, *E. coli*, even at 1000 µg/disc concentration.

In contrast, only the combined extract exhibited antibacterial activity against Gram positive bacteria, *S. aureus* at 500 and 750 µg/disc concentrations. A 1000 µg/disc concentration, the fruit extract (2.93 ± 0.03mm), stem bark extract (5.1 ± 0.03mm) and the combined extract (9.46 ± 0.03mm) exhibited respectively significant (P<0.00) mild, moderate and strong antibacterial activities. Furthermore, the antibacterial activity of the combined extract was dose-dependent (r^2=, 0.97 P<0.05).

The reference drug, Gentamicine, provoked a significant (P<0.05) and marked antibacterial activity against both *S. aureus* (13.92 mm), *E. coli* (15.02mm).

Table 1: *In vitro* antibacterial activity of water extracts of different parts of *Morinda citrifolia* against *Staphylococcus aureus* and *Escherichia coli*

Plant Extract	Antibacterial activity (inhibition Zone diameter – mm) *S.aureus* Strains (ATCC 25923)				*E.coli* Strains (ATCC 35218)		
	500 µg/disk	750 µg/disk	1000 µg/disk	r^2 Value	500 µg/disk	750 µg/disk	1000 µg/disk
Roots	-	-	-	-	-	-	-
Fruits	-	-	2.93 ± 0.03	-	-	-	-
Flowers	-	-	-	-	-	-	-
Leaves	-	-	-	-	-	-	-
Stem Barks	-	-	5.1 ± 0.03	-	-	-	-
Combined Sample	5.06 ± 0.03	6.56 ± 0.03	9.46 ± 0.03	0.97	-	-	-
Gentamicin	13.86 ± 0.02	13.92 ± 0.03	13.98 ± 0.04	N/A	15.00 ± 0.02	15.02 ± 0.03	15.04 ± 0.02
Distilled water	0.00	0.00	0.00	N/A	0.00	0.00	0.00

Data presented as mean Inhibition zone diameter ± SEM (n=3).
Sign (-) indicates no antibacterial activity. Sign (N/A) indicates not applicable.
Gentamicin was used as the Positive control. Distilled Water was used as the Negative control.

IV. Discussion

This study examined the antibacterial potential of aqueous extracts of (roots, fruits, flowers, leaves, stem bark and combination of these parts) *M. citrifolia in-vitro* with a view of providing scientific justification of its use as a topical antibacterial agent to wash old cutaneous wounds, cuts abrasions, bruises or burns, and as a throat gargle, in traditional and folk medicine in Sri Lanka [4, 5]. We used hot water extracts in testing the antibacterial activity because hot water extracts of various parts of the plant are generally used in traditional and folk medicine. Moreover, in testing for pharmacological activity of a traditionally used phytomedicines it is generally recommended to use water, in the same manner as the traditional medicine is made, although methonolic extracts are used widely in the evaluation of antibacterial activity of herbal extracts *in vitro* [8, 9, 10]. Two commonly encountered human pathogenic bacterial species infecting skin wounds, one Gram positive (*S. aureus*) and one Gram negative (*E. coli*), [6], and agar disc diffusion bioassay was used in assessing the antibacterial activity in this study. This is a reliable, sensitive and widely used technique in the evaluation of the antibacterial activity of plant medicines [8, 9, 10, 11, 12].

The results show, for the first time, that water extracts made from fruits, stem bark and combination of parts of *M. citrifolia* grown in Sri Lanka, has mild to moderate antibacterial activity against Gram positive *S. aureus* and absolutely no antibacterial activity against Gram negative *E. coli*, at the concentration tested. Most interestingly, this the second study to show that a water extract of M. *citrifolia* has promising antibacterial activity although several other studies have previously shown antibacterial activity using organic extracts (methonolic, ethonolic, ethyl acetate) of fruits, leaves, seeds and stems of this plant [1, 2, 3, 9, 11, 13, 14]. In some of these studies [1, 2, 3, 13], in compliance with our study, and not with others [9, 11, 13] *M. citrifolia* was found to be more effective against Gram positive bacteria (eg. *S.aureus*) than Gram negative bacteria (eg. *E. coli*). Generally, Gram negative bacteria are less susceptible to herbal extracts and antibiotics [1, 2, 3, 8, 15] as their membrane of lipoproteins and lipopolysaccharides impairs the access of antibacterial agents into underlying structures [7]. The general order of potency of the antibacterial activity evident in this study was, combinations of parts > stem bark > fruits. The highest antibacterial activity of the extracts made from combination of parts is likely to be due to a synergistic activity and/or due to the formation of a new product during the preparation of the whole extract using roots, fruits, flowers, leaves, stem bark, and the verification of these two modes of actions will be examined in future studies. Further, as a whole, in this study, the antibacterial activity of water extracts of different parts and combination of parts of *M. citrifolia* against *S. aureus* was lower than reported by some others [1, 2, 3, 13]. This may be due to differences in solvents used in the extraction procedure, strains of bacterial species, concentrations and techniques used in testing the antibacterial activity. Alternatively, it could be due to differences in chemical composition and concentrations of phytoconstituents which are known to vary from country to country, harvesting periods, or on climatic conditions [2].

Although, we have not investigated the phytochemical profile of *M. citrifolia* extracts, almost 200 phytochemicals are identified and isolated from different parts of this plant. These include tannins, flavonoids, phenolic, alkaloids, terpernoids, saponins, lipids, steroids or sugars [1, 2]. Of these, only hydrophilic polar phytocomstituents are extracted in to water, which are likely to provoke the antibacterial action evident in this study, possibly via disrupting the structural and functional integrity of plasmalemma [8] or by interacting with bacterial DNA [8].

V. Conclusion

This study shows, for the first time, marked antibacterial activity of water extracts of fruits, stem bark and combination of different parts of *M. citrifolia* grown in Sri Lanka. In addition, it also provides scientific justification for the use of water extracts of this plant as a topical antibacterial for washing and cleaning of old cutaneous cuts, bruises, abrasions, rashes and burns in traditional and folk medicine of Sri Lanka.

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