ANTIBACTERIAL EFFECTS OF THAI MANGO (*Mangifera indica* Linn.) LEAVES AGAINST ACNE-INDUCING BACTERIA

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ABSTRACT: *The properties of mango (Mangifera indica Linn.) leaf extracts have been widely studied in many countries. However, until now, the antibacterial activity of Thai mango leaves against acne-inducing bacteria has not been reported. The objective of this study was to ascertain the antibacterial activity of methanolic and aqueous extracts from the leaves of three M. indica varieties, Keaw Morakot (KM), Nam Doc Mai (NM) and Mahajanaka (MN) by agar disc diffusion and broth dilution assays. The results from the disc diffusion assay showed that leaf extracts from all tested M. indica varieties could inhibit the growth of Staphylococcus epidermidis, Staphylococcus aureus, methicillin-resistant Staphylococcus aureus (MRSA), Propionibacterium acnes and Pseudomonas aeruginosa. Among those, the methanolic extracts of M. indica leaves had the strongest inhibitory effects. Based on the broth dilution assay, the methanolic extract of MN had the greatest antibacterial effect. The minimal inhibitory concentration (MIC) values were 3.91, 7.81, 15.63, 62.5 and 125 mg/ml for S. epidermidis, S. aureus, MRSA, P. acnes and Ps. aeruginosa and the minimal bactericidal concentration (MBC) values were 7.81, 15.63, 31.25, 125 and 250 mg/ml, respectively. Therefore, the leaves of Thai mangoes require further study because they could possibly be used as an alternative acne treatment.*

Keywords: antibacterial activity, *Mangifera indica* Linn., leaf extract, acne

1. INTRODUCTION

Propionibacterium acnes (*P. acnes*) is the causative agent of the chronic skin infection, acne vulgaris. The infection may lead to inflammation of the skin. One way to successfully treat acne is to reduce the bacteria using topical antibiotics such as tetracycline, erythromycin and clindamycin [1]. Nevertheless, a long time use of these chemicals may cause many undesirable effects; i.e., skin irritation, organ damage, immune hypersensitivity and bacterial resistance [2-3] and [4]. Due to the high cost and the various side effects of synthetic drugs, exploitation of medicinal plants as an alternative treatment for acne has greatly increased in recent years. Generally, many plants have been consumed for health benefits or beauty such as *Senna alata*, *Eupatorium odoratum*, *Garcinia mangostana* and *Barleria lupulina* [5]. Moreover, mango (*Mangifera indica* Linn.) extract has become one of the ingredients for cosmeceutical products.

M. indica (Anacardiaceae) grows in the tropical and subtropical region and its parts are commonly used in folk medicine for various remedies [6]. Pharmaceutical information about *M. indica* leaves from other countries has been well documented, Chinese *M. indica* leaves have a potential for development as an anti-gouty arthritis agent for clinical application [7]. *M. indica* leaf extracts from India can also be potentially used in pharmaceutical applications as chemopreventive agents of diseases related to oxidative stress given that their numerous antioxidant and health-promoting properties have been scientifically demonstrated [8]. However, the biological activity of Thai *M. indica* leaves has not been reported. Therefore, we are interested in the study of the antibacterial activity of Thai *M. indica* leaf extracts from three varieties of mangoes grown in Thailand, Keaw Morakot, Nam Doc Mai and Mahajanaka were selected due to the differences in cultivated area, leaf characteristics, and genetic diversity.

2. EXPERIMENTAL DETAILS

2.1 Plant materials and extraction

Three varieties of *M. indica* leaves were collected from Ban Hong District, Lamphun Province, Thailand during March and April, 2014 (Figure. 1). The plant species and varieties were identified by a botanist of the Queen Sirikit Botanic Garden, Mae Rim, Chiang Mai, Thailand Voucher specimen of Mahajanaka (MN), Nam Doc Mai (NM) and Keaw Morakot (KM) are WP 5059, WP 5060 and WP 5061 respectively. The leaves were extracted by two solvents (methanol and distilled water). Ground dry leaves of mango were macerated in both solvents for 24 hours at room temperature. Each extract was filtered with Whatman No.1 and the solvents were removed by vacuum rotary evaporator. Then the crude extracts were lyophilized to obtain a crude powder. Finally, the extracts were dissolved in dimethyl sulfoxide (DMSO) before determination of antibacterial activity.

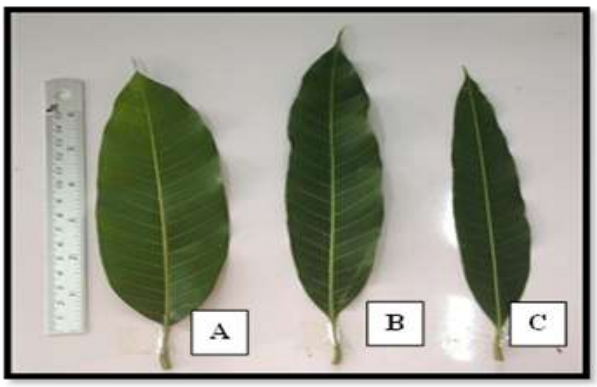

Figure (1) Three varieties of *M. indica* leaves (A: Keaw Morakot, B: Nam Doc Mai, C: Mahajanaka)

2.2 Phytochemical analysis

Mango leaf extracts were determined for ten phytochemicals (terpenoids, alkaloids, anthraquinones, flavonoids, saponins, tannins, steroids, phenolics, reducing sugar and cardiac glycosides) by following the methods of Harborne [9], Peach and Tracey [10], Edeoga et al. [8], Kasolo et al [11] and Arunachalam and Chinnaraju [1].

2.3 Agar disc diffusion assay

The tested bacterial strains (*Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, methicillin-resistant *Staphylococcus aureus*; MRSA and *Staphylococcus epidermidis* ATCC 14990) were cultured in Mueller-Hinton Broth (MHB, Merck®) at 37°C for 18-24 hours. The turbidity of the culture was adjusted to yield approximately 1.0 x 10^8 CFU/ml. Each culture was swabbed on Mueller-Hinton Agar (MHA, Merck®). Then a sterile paper disc (Macherey-NagelTM) with 0.6 cm diameter was soaked in 500 mg/ml crude extracted solution and placed on the agar. After that, it was compared with the control (DMSO). Plates were incubated at 37°C for 24 hours [12]

Propionibacterium acnes DMST 14916 was cultured in Brain Heart Infusion Broth (BHI, Merck®) at 37°C for 72 hours and adjusted to yield approximately 1.0 x 10^8 CFU/ml. The next procedures were performed as above, except the plates were incubated at 37°C for 72 hours under anaerobic conditions. After the incubation period, diameters of the inhibition zone were measured to access antibacterial activity. All experiments were performed in triplicate and means of inhibition zone were calculated [12].

2.4 Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) was measured by the broth dilution method. Two-fold serial dilutions of crude extracts were prepared with 0.5 ml MHB or BHI before inoculating the bacteria with 0.5 ml of bacterial cultures. Test tubes were incubated at 37°C for 72 hours under anaerobic conditions for *P. acnes* and at 37°C for 24 hours for other bacteria. MIC was recorded as the lowest concentration of crude extracts in which the growth was inhibited. For the MBC evaluation, the tubes with no bacterial growth were streaked on MHA or BHI agar plates and incubated under the above conditions for different bacterial strains. The MBC was recorded as the lowest concentration showing no visible growth of bacteria [12].

2.5 Statistical Analysis

For statistical analysis, data were analyzed by one-way analysis of variance (ANOVA) using SPSS statistical software version 17.0 for windows. The results were expressed as a mean ± standard deviation. A level of p-value less than 0.05 was considered to be significant.

Table 1. Phytochemical screening of methanolic and aqueous extracts of *M. indica* leaves

Test	*M. indica* leaves					
	Methanolic extracts			**Aqueous extracts**		
	KM	NM	MN	KM	NM	MN
Terpenoids	+	+	+	+	+	+
Alkaloids	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Steroids	-	-	-	-	-	-
Phenolics	+	+	+	+	+	+
Reducing sugar	+	+	+	-	-	-
Cardiac glycosides	-	-	-	-	-	-

Key: +: Present; -: Absent.

Table 2. Inhibition zones of tested bacteria after treatment with *M. indica* leaf extracts

M. indica leaves	Zone of inhibition (mm in diameter; mean ± SD; n = 3)				
	S. epidermidis	*S. aureus*	MRSA	*P. acnes*	*Ps. aeruginosa*
Methanolic extracts					
Keaw Morakot	20.50±2.35^{a}	16.83±0.98^{a}	16.67±1.97	13.33±2.73	10.00±1.10^{a}
Nam Doc Mai	20.00±3.90^{ab}	17.33±0.52^{a}	17.00±2.37	13.67±3.39	11.50±1.52^{ab}
Mahajanaka	18.83±1.33^{abc}	16.33±1.03^{ab}	17.33±2.16	14.50±4.13	09.83±0.41^{a}
Aqueous extracts					
Keaw Morakot	13.67±4.23^{bc}	13.17±2.79^{b}	14.33±3.01	10.33±2.80	11.33±0.82^{ab}
Nam Doc Mai	13.33±5.20^{c}	13.00±2.45^{b}	13.50±2.59	12.00±5.22	12.83±2.14^{b}
Mahajanaka	15.17±3.92^{abc}	14.50±2.81^{ab}	13.50±2.07	11.83±2.99	11.67±1.63^{ab}

$^{a, b, c}$ Means in the same column with different superscripts are significantly different at $P<0.05$.

3. RESULTS

This study of *M. indica* leaf extracts revealed the presence of terpenoids, flavonoids, saponins, tannins, phenolics and reducing sugar in the methanolic extracts and terpenoids, flavonoids, saponins, tannins and phenolics in the aqueous extracts. The leaves did not show the presence of alkaloids, anthraquinones, steroids and cardiac glycosides in any of the extracts that were tested (Table 1)

To elucidate the antibacterial activity of *M. indica* leaf extracts against acne-inducing bacteria, we introduced a disc diffusion method (Table 2). Gentamicin was used as a positive control and DMSO was used as a negative control. Methanol and distilled water extracts of *M. indica* leaves inhibited all tested bacteria. When using methanol as the extraction solvent, the leaves of three *M. indica* varieties, Keaw Morakot, Nam Doc Mai and Mahajanaka, had the inhibitory properties against the gram-positive bacteria more than the gram-negative bacteria.

The methanolic extract of Keaw Morakot showed the highest inhibitory effect against *S. epidermidis* with an inhibition zone of 20.50±2.35 mm, while the highest inhibition zones of 14.50±4.13 and 17.33±2.16 mm were observed against P. acnes and MRSA after testing with the methanolic extract of Mahajanaka. The methanolic extract of Nam Doc Mai exhibited the highest inhibitory effect against *S. aureus* with an inhibition zone of 17.33±0.52 mm. When using distilled water as an extraction solvent, Nam Doc Mai showed inhibition zone of 12.83±2.14 mm against *Ps. aeruginosa*, which had the highest activity compared to other tested bacteria (Table 2 and Figure 2)

Subsequent experiments were conducted to determine the inhibitory concentrations of all extracts. The methanolic extract of Mahajanaka showed the greatest antibacterial effect. The MIC values against *S. epidermidis*, *S. aureus*, MRSA, *P. acnes* and *Ps. aeruginosa* were 3.91, 7.81, 15.63, 62.5 and 125 mg/ml, respectively and the MBC values were 7.81, 15.63, 31.25, 125 and 250 mg/ml, respectively (Table 3

)

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values (mg/ml) of *M. indica* leaves against acne-inducing bacteria

M. indica leaves	Bacterial strains									
	S.epidermidis		*S. aureus*		MRSA		*P. acnes*		*Ps. aeruginosa*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Methanolic extracts										
Keaw Morakot	7.81	7.81	15.63	15.63	15.63	31.25	62.5	125	125	250
Nam Doc Mai	3.91	7.81	15.63	15.63	15.63	31.25	62.5	125	125	250
Mahajanaka	3.91	7.81	7.81	15.63	15.63	31.25	62.5	125	125	250
Aqueous extracts										
Keaw Morakot	7.81	15.63	15.63	31.25	31.25	62.5	125	125	250	250
Nam Doc Mai	7.81	15.63	31.25	31.25	31.25	62.5	125	125	250	250
Mahajanaka	15.63	15.63	31.25	31.25	31.25	62.5	125	125	250	250

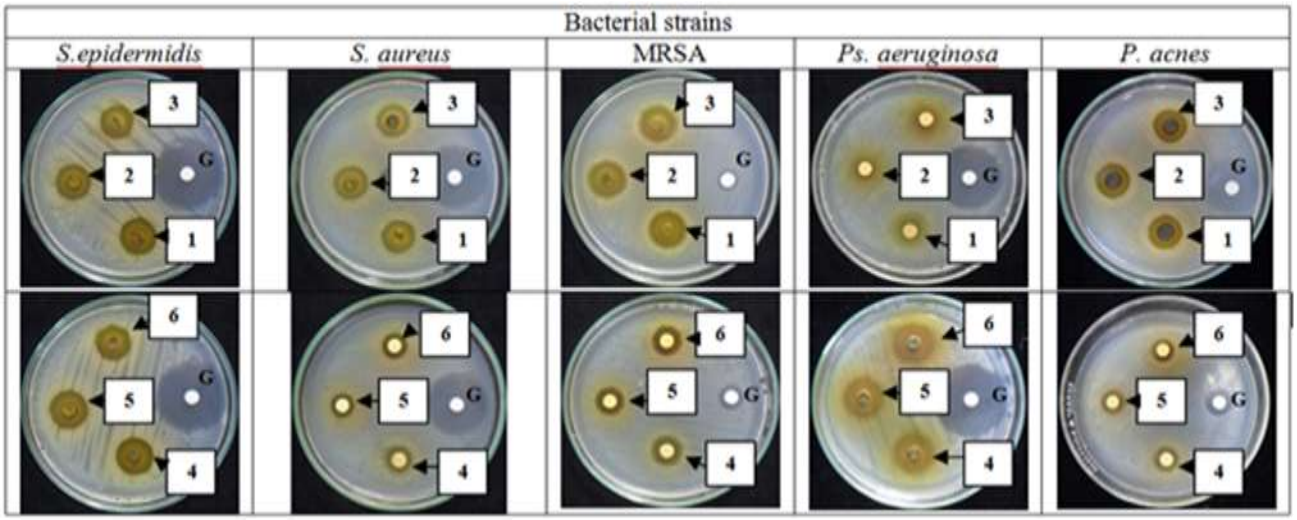

Figure 2. Anti-bacterial activity of methanolic and aqueous extracts of *M. indica* by agar disc diffusion assay (1: Keaw Morakot-Methanolic extract, 2: Nam Doc Mai- Methanolic extract, 3: Mahajanaka-Methanolic extract, 4: Keaw Morakot-Aqueous extract, 5: Nam Doc Mai- Aqueous extract, 6: Mahajanaka- Aqueous extract, G: Gentamicin 2.5 mg/ml)

4. DISCUSSION

There are several reports comparing bioactive compounds of different varieties of the same plant species. Differences or similarities can potentially be used to explain variations in pharmacological activity [11, 13-15]. Characteristic of three varieties of *M. indica* leaves (KM, NM, MN) based on macroscopic, microscopic, and genetic characters [16].

In this study, different varieties of Thai *M. indica* leaves, Keaw Morakot, Nam Doc Mai and Mahajanaka showed antibacterial effect, especially the methanolic extracts. *M. indica* leaves, which have been reported for their potency to inhibit bacteria, exerted antibacterial activity against both Gram-positive and Gram-negative bacteria. The previous study showed the activity of the methanolic extract of *M. indica* leaves against bacteria that was greater than other solvents extract [10, 17].

The methanolic extracts had greater inhibitory properties against the Gram-positive bacteria than the Gram-negative bacteria which is attributed to the fact that the cell wall in Gram-positive bacteria is single-layered of thick peptidoglycan, whereas Gram-negative cells are multilayered. The outer membrane found in the Gram-negative cell wall is composed of structural lipopolysaccharides which render the cell wall impermeable to lipophilic solutes. The antibacterial activities of methanolic extracts were found to be more potent than those of aqueous extracts. Moreover, Gram-positive bacteria are already known to be more susceptible to plant extracts than Gram-negative bacteria [18-19].

The presence of phytoconstituents, such as flavonoids, tannins, coumarins, alkaloids, terapenoids and phenolics in the leaf extracts may be responsible for the antibacterial activity of the plant [20-21]. The differences in the observed activities of the various extracts may be due to the varying degrees of solubility of the active constituents in the two solvents used. It has been documented that different solvents demonstrated diverse solubility capacities for different phytoconstituents [22].

Bacteria used in this study are associated with acne vulgaris. The low MIC and MBC values against these bacteria meant that the Thai mango leaves in this study had the potential to treat acne effectively. The leaves and kernel of *M. indica* from other countries have been reported to possess antibacterial activity against Gram-positive (*S. aureus*) and Gram-negative (*Ps. aeruginosa*) bacterial strains [18, 23]. The major constituent of *M. indica* leaves is Mangiferin (C-2-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone, also named C-glucosyl xanthone), which possesses various pharmacological activities [24]. Singh et al. [22] reported the solutions of mangiferin and its derivatives from *M. indica* showed a wide range of effects both with regard to Gram-positive as well as Gram-negative bacteria. However, mangiferin and its derivatives, when used at high concentrations, exerted an antibacterial effect against Gram-negative microorganisms. Mangiferin did not show any activity against *Ps. aeruginosa* while its derivatives showed activity at high concentrations only [25].

The present study confirmed the antibacterial effects of Thai mango leaves. Further investigations of this plant will be performed by identification and purification of its chemical constituents. Antioxidant and anti-inflammatory properties will be investigated. The experiments should be carried out with a view to developing novel drugs for acne treatment.

5. CONCLUSIONS

This study is the first study to demonstrate that the leaves of three *M. indica* varieties cultivated in Thailand: Keaw morakot, Nam Doc Mai and Mahajanaka possess antibacterial activity against acne-inducing bacteria.

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