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ANTIBACTERIAL EFFECTS OF ETHANOLIC LEAF EXTRACTS OF BACHANG (*MANGIFERA FOETIDA* LOUR.) ON *STREPTOCOCCUS MUTANS*

Lilik Koernia Wahidah[1], Ahmad Rokiban[1], Subur Widodo[1], Dewi Mut Mainah[1], Yulianty[2] and Mohammad Kanedi[2]*

[1]Department of Pharmacy, Faculty of Mathematics and Sciences, Tulang Bawang University, Bandar Lampung, Indonesia.

[2]Department of Biology, Faculty of Mathematics and Sciences, University of Lampung, Bandar Lampung, Indonesia.

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***Corresponding Author**
Dr. Mohammad Kanedi
Department of Biology, Faculty of Mathematics and Sciences, University of Lampung, Bandar Lampung, Indonesia.

ABSTRACT

Bachang (*Mangifera foetida* Lour.) get less attention in scientific studies, so that it is difficult to find literature about the benefits of this mango in the folk medicine practice and contemporary pharmacology. The study was carried out to find out if the leaf extract of *M. foetida* is potent as antibacterial against *Streptococcus mutans*, the dental caries-causing bacteria. Seven different solution were prepared for treatments namely aquadest (as negative control), erythromycin (as positive control) and five levels concentration of bachang leaves extract (v/v), ie 20%, 40%, 60%, 80% and 100%. Susceptiblity of the bacteria was assayed by disc diffusion technique with blood agar base. Minimum inhibitory concentration(MIC) and minimum bactericidal concentration (MBC) were determined by a serial dilution technique with nutrient broth media were used. The results showed bachang leaf extracts significantly inhibit the growth of *S.mutans* with inhibition zones ranging from 13.74 mm to 19.22 mm. At the maximum concentration the effect even the same as erythromycin. With the MIC value of 14%, the extract exhibits bactericidal properties by MBC test.

KEYWORDS: Bachang, *Mangifera foetida*, wild mango, mangiferin, *Streptococcus mutans*, antibacterial.

INTRODUCTION

Bachang (*Mangifera foetida* Lour.) is one of the mango species native to Indonesia that possesses particularities on its fruit. Unripe bachang fruit contain an irritant juice which decrease at maturity and left the irritant sap restricted to the peel that make the fruit flesh can be eaten fresh. In Indonesia, this mango generally intercropped with other crops in plantations. In addition, in spite of its turpentine smell and the taste but it is not generally valued as a table fruit and less marketable.[1]

Due to less commercial, presumably, causing bachang get less attention in scientific studies, so that it is difficult to find literature about the benefits of this mango in the folk medicine practice and contemporary pharmacology. In contrast, the scientific works on various aspects of agronomic, biochemical, pharmacological, medicinal and health benefits of Indian mango (*Mangifera indica* L.), the most valuable manggo in the world, are very abundant. Indian mango, the common mango, in each home land of the plant is known to have numerous medicinal uses namely to treat diarrhea, cough, anemia, hypotension, itch, diuretic, rheumatism, gingivitis, dysentery, syphilis, emetic, gastric disorders, hepatic disorders, bleeding hemorrhoids, lunghemorrhage, diabetes, menorrhagia, jaundice, liver obstruction, wounds, mouth sores, toothache, chest pain, anemia, skin diseases and dental caries.[2]

Today *M. indica* suggested as an important source of many pharmacologically and medicinally important chemicals such as mangiferin, mangiferonic acid hydroxymangiferin, polyphenols and carotenes. Mangiferin, named based on the generic name of the plants, exhibits many different pharmacological activities, including antioxidant, radioprotective, immunomodulatory, anti-allergic, anti-inflammatory, antitumor, antidiabetic, lipolytic, antiboneresorption, monoamine oxidase-inhibiting, antimicrobial and antiparasitic.[3] In *M. indica*, the mangiferin can be isolated from leaves, stem bark, fruit peels and root, peels of raw and ripe fruits, fruit peel and pulp.[4]

Among the few studies on *Mangifera foetida*, known as wild mango, show that this mango fruits containing several volatiles substances such as, esters and oxigenated monoterpenes were dominant, with ethyl butanoate the most abundant.[5] A study on antioxidant properties of fresh, powder and fiber products of *M. foetida* fruit showed that bachang fruit containing flavonoid, carotenoid and ascorbic acid which exhibited antioxidant properties.[6] A more recent study using phytochemical tube assay and TLC (thin layer chromatography) analysis

showed that the ethanol fraction of the wild mango's leaf extract contained phenols, coumarins, flavonoids, tannins, alkaloids and quinones.[7]

The existence of flavonoids in *M. foetida* can be used as a basis to expect that the crude extract of this mango will exhibit antibacterial properties. There are lots of research reports on the pharmacological effects of a plant that points flavonoids as active substance. Flavonoids isolated from leaves of Indian mango (M. indica L.) is known to inhibit the growth of *Lactobacillus* sp., *Escherichia coli*, *Azospirillium lipoferum* and *Bacillus* sp.[8] Bioactive substances isolated from leaves and stems of Chromolaena squalida that evidently shown antibacterial activities on both Gram-positive and Gram-negative bacteria known to contain flavonoids.[9]

In spite of common mango plant (*M. indica*) has been used for treating dental caries and the mango plant extract in fact revealed antibacterial activities, yet there is no report regarding the effects of the mango plant extract on the bacteria causing dental caries. For that reason, the study was carried out to find out if the leaf extract of *M. foetida* is potent to inhibit the growth of *Streptococcus mutans* or even possesses bactericidal effects against the bacteria..

MATERIALS AND METHODS

Bachang Leaves Extract

Bachang mango leaves used in the study were collected from Karangsari village, sub-district of Air Naningan, the district of Tanggamus, Lampung province, Indonesia. The leaves that were chosen as a sample is the third, fourth, fifth, sixth and seventh leaf from the shoot end of a twig. Once washed with tap water and rinsed with distilled water, the fresh leaves were chopped into small pieces and then sun-dried after being covered with a black cloth. The simplicia (100 g) were macerated using 70% ethanol for 24 hours, repeated three times. The macerate then evaporated using a rotary evaporator until concentrated liquid extracts were obtained and labelled as stock solution. The stock solution was diluted with distilled water in accordance with the treatment concentrations designed for the experiment.

Experimental Design

By using a completely randomized design, seven different solution were prepared for treatments in the study namely aquadest (as negative control), erythromycin (as positive control) and five levels concentration of bachang leaves extract (v/v), ie 20%, 40%, 60%, 80% and 100%. Each treatment was replicated three times.

Bacterial Inhibition Test

The disc diffusion assay technique was used, 100 µl of microbes cultures aged 18-24 h were add to Petri disc and blood agar base (BAB) were poured. After media were solidified, the disc (with a diameter of 5 mm) that have been previously soaked in treatment solution were placed. The inoculated agar plates incubated at 37^{o}C for 24 h. The zones of inhibition were then recorded in millimeters.

Determination of MIC and MBC

The minimum inhibitory concentrations (MIC) of the bachang leaves extracts performed by a serial dilution technique with nutrient broth (NB) media were used. The serial dilution concentrations of the stock were 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11% , 12%, 13%, 145, 15%, 16%, 17%, 18% and 19% along with three control solution, ie media, plant extract and inocula. The media control contained 2ml NB; extract control containing 1.8 ml NB and 0.2ml plant extract; while inocula control containing 1.9ml NB and 0.1ml bacterial suspension. The minimum inhibitory concentration was defined as the lowest concentration able to inhibit any visible bacterial growth after being incubated for 24 hours. To determine the minimum bactericidal concentration (MBC), bacterial suspension in the extract solution set as MIC were re-cultured on Mueller-Hinton Agar and incubated for 24 hours. The highest dilution that yielded no single bacterial colony was taken as the minimum bactericidal concentration.

Statistical Analysis

Both descriptive and inferential statistics were used. Statistical differences between groups were analyzed by one-way analysis of variance (ANOVA) followed by LSD test. The data were presented as the mean±SD and the statistical significance was established at $p < 0.05$.

RESULTS

The susceptibility and resistance of *S. mutans* against the aquadest (as negative control), bachang leaf extracts and erythromycin (positive control) treatments are presented in Table 1. One-way ANOVA statistics applied for the mean value of the inhibition zone diameters resulted in *F-value* of 183.527 (F-crit of 2.848) and *P-value* < 0.001. The data clearly indicated that ethanolic leaf extracts of the bachang mango (*M. foetida* Lour.) of all concentration levels are significantly differ from aquadest (the negative control) and at the highest concentration (100%) the effect even the same as erythromycin (the positive control).

Table 1. The results of disc diffusion test against the effects of aquadest (as negative control), bachang leaf extracts and erythromycin (positive control) on the growth of *S. mutans*.

Treatments	Diamater of Inhibition Zones (mm)			Mean ± SD
	1	2	3	
Aquadest	0	0	0	0^{a}
Bachang 20%	12,2	15,2	13,8	$13,74±1,52^{b}$
Bachang 40%	14,4	17,3	16,8	$16,15±1,58^{cd}$
Bachang 60%	16,5	16,1	14,3	$15,61±1,20^{c}$
Bachang 80%	17,6	17,1	18,1	$17,61±0,50^{d}$
Bachang 100%	19,8	18,7	19,3	$19,22±0,55^{de}$
Erythromycin	20,2	19,4	19,6	$19,72±0,39^{e}$
Mean±SD values followed by the same superscript are not statistically different at α=0.05, based on the LSD test result.				

The result of MIC test against *S.mutans* inocula using a series of dilution concentration of extract along with the control solution, are tabultaed in Table 2. It is clearly indicated by the data that there is no bacterial growth visible in the test tube contained leaf extracts of bachang 19% -14%. It is suggested, therefore, that 14% is the minimum inhibitory concentration of ethanolic leaf extracts of bacang mango against the growth of *S.mutans*.

Table 2. Results of MIC test of bachang leaf extracts against the growth of *S.mutans*

Test tube No.	Concentration of solution	Bacterial growth
1	19%	-
2	18%	-
3	17%	-
4	16%	-
5	15%	-
6	14%	-
7	13%	+
8	12%	+
9	11%	+
10	10%	+
11	9%	+
12	8%	+
13	7%	+
14	6%	+
15	5%	+
16	4%	+
17	3%	+
18	2%	+
19	1%	+

20	Media control	-
21	Extract control	-
22	Bacterial control	+
(-): no bacterial growth visible (+): bacterial growth occurs		

The results of re-culturing test on the growth of *S.mutans* suspension taken from MIC solution series (12% - 15%) on BAB agar are depicted by photographs in Fig.1. The photographs showed that bacterial inocula taken from MIC solution of 14% and 15% yielded no bacterial colony at all. It can be assumed that the ethanolic leaf extracts of *M. foetida* possess bactericidal properties, instead of bacteriostatic, against *S.mutans*.

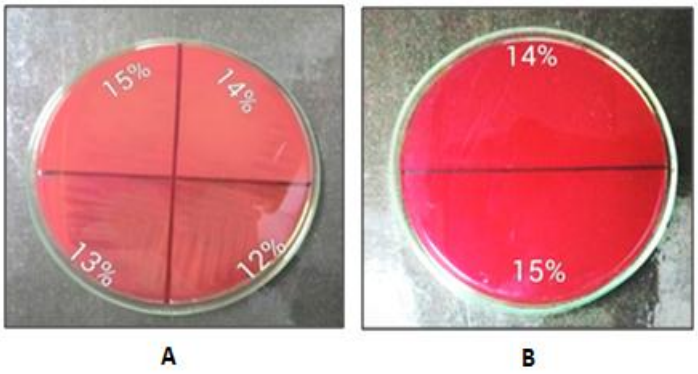

Figure 1. Photographs depicted the growth of *S.mutans* inocula taken from solution used in MIC test. (A) Comparison between the concentration in the MIC test where bacterial growth was visible (12% and 13%) and that was not (14% and 15%); (B) Growth of *S. mutans* taken only from the dilution concentration that does not show bacterial growth.

DISCUSSION

As explained in the introduction, this plant contains active ingredients such as esters and monoterpenes, coumarins, tannins, alkaloids, flavonoid, carotenoid, ascorbic acid, phenols, and quinones. Some of these chemicals have been reported to have anti-bacterial properties. Monoterpenes with its active components such as (+)menthol, thymol and linalyl acetate was suggested to result in alterations of membrane permeability and in leakage of intracellular materials of bacterial cells.[10]

Coumarins and the derivatives such as osthenol showed effective antibacterial activity against *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* with MIC values ranging between 125 and 62.5 µg/ml. The prenyl chain and OH component of

osthenol was suspected to accoun for antibacterial activities of this molecule against these strains.[11] Next, tannins. Purified tannin extracts from tannin-containing plants such as oak, locust, skunk bush and plum exhibited a range of antimicrobial activity against *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus*.[12]

Among bioactive extracts from *M. foetida* mentioned above, flavonoid is the most suggested as the highly potent antibacterial. Methanolic leaf extracts from guava plant (*Psidium guajava*), which known to contain flavonoids, were suggested as antibacterial due to cause significant release of RNA in gram-negatives and gram-positives bacteria. The guava extracts was allegedly disrupt the integrity of the microorganism lipopolysaccharide (LPS) layer.[13] The disruption of bacterial membrane by flavonoid treatments has also been confirmed by more recent studies. Dzoyem et al.[14] found that flavonoids from *Dorstenia* species lead to depolarization of membrane and inhibition of DNA, RNA and proteins synthesis in *S.aureus*, causing the decrease of cell density and lysis of the bacteria. Quercetin, one of flavonoid derivatives, attributed to inhibition of DNA gyrase, while the other derivates such as sophoraflavone G and (−)-epigallocatechin gallate inhibit cytoplasmic membrane function, and that licochalcones A and C inhibit energy metabolism.[15] Lipophilic flavonoids found to disrupt microbial membranes; catechin inhibit isolated bacterial glucosyltransferases in *S. mutans*; robinetin, myricetin and (−)-epigallocatechin are known to inhibit DNA synthesis in *Proteus vulgaris*.[16]

The last, due to Mangifera species were the main sources of mangiferin, it is appropriate to assume that the antibacterial effects of *M.foetida* might related to mangiferin. In Fusarium wilt of safflower, mangiferin caused lysis of the hyphal cells and reduced growth and presumably, also altered the metabolism of the fungus.[17] Mangiferin was considered as an antimicrobial agent upon gram-positive, gram-negative bacteria and yeast *Candida albicans* and it was a helping agent for up-regulating the multidrug transporter of ABCB1/Pglycoprotein.[18]

CONCLUSSION

Ethanolic leaf extracts of bachang mango (*Mangifera foetida* Lour.) possess antibacterial and bactericidal properties against *Staphylococcus mutans,* the dental caries-causing bacteria.

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