

Short Communication

In-Vitro Antibacterial Properties of Ethanol and Methanol Extracts of Betel Leaves Collected from Different Areas of Bangladesh

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Current investigation depicted *in-vitro* antibacterial activity of available betel leaf (sweet, hot and fresh betel leaves) collected from different place of Bangladesh. The *in-vitro* antimicrobial activities of the ethanol and methanol extracts of the betel leaf were noticeable against tested bacteria. Among 3 categories of samples, the antibacterial action of ethanol extracts of hot betel leaf was found the highest against most of the bacteria except *Klebsiella* sp. and *Staphylococcus* sp., while the methanol extract showed antibacterial activity against all the pathogenic strains tested. The highest MIC (1mg/ml) and the lowest MIC values (25mg/ml) of hot betel leaf and sweet betel leaf were found against *E. coli* and all test organisms, respectively. This evidence revealed that betel leaf extracts can be used to combat against human pathogens.

Keywords: Antibacterial properties, betel leaf.

Introduction

The increasing resistance phenomenon of infectious agents to various antimicrobial products has revealed the importance of developing new drugs using varied strategies such as – bio-prospection of secondary metabolites of medicinal plants¹⁻⁶. At present, it has become difficult to treat diseases due to the increasing manifestation of multiple resistants⁷. Biologically, betel leaf is aromatic, stimulant, carminative, astringent and antiseptic⁸. They are widely consumed as a mouth freshener in Southeast Asia and rank second to coffee and tea in terms of daily consumption. The leaves of betel are chemo-preventive and used to treat alcoholism, bronchitis, asthma, joint pain, itchiness, leprosy and dyspepsia⁹⁻¹¹. Betel leaves can act as high antioxidant and it contains hydroxychavicol, eugenol, ascorbic acid and β -carotene^{12,13}. Scientifically, studies have reported the biological benefits of betel leaf to include anti-diabetic activities¹⁴, and anti-allergic activities¹⁵. Antibacterial properties of ethanol extract of betel leaf against food borne pathogens were reported by Hoque *et al*¹⁶. Along these lines, present study endeavoured to launch *in-vitro* antibacterial properties of betel leaf.

Materials and Methods

Study Area, Sampling, Sample Processing and Microbiological Analysis

Total 15 samples of three categories of betel leaves including sweet betel leaf, hot betel leaf and fresh betel leaf were randomly collected following standard protocol¹⁷.

Test organisms

E. coli, *Klebsiella* sp., *Salmonella* sp., *Bacillus* sp., *Listeria* sp., *Pseudomonas* sp. *Vibrio* sp. and *Staphylococcus* sp. were tested for antibacterial activity of the betel leaf extracts.

Solvent extraction

Each of the betel leaves were dried and grounded properly for ethanol, methanol and hot water extraction. Afterward 10g of well grind powder were added to 90 ml of ethanol and methanol in Durham's bottle similarly same amount of powder was added in 140 ml of hot water then all the samples were kept in shaking water bath at 130 rpm for 24 h. at 20 °C¹. All the extracts were then filtered to separate the pellet and supernatant. After that the liquid substances were collected to perform the anti-bacterial activity through agar well diffusion methods.

Assay of antimicrobial activity

The Mueller-Hinton agar (MHA) plates were prepared followed by Modified agar well diffusion method^{18,19}. Total 8 bacterial suspensions including *Escherichia coli*, *Pseudomonas* sp., *Listeria* sp., *Vibrio* sp., *Klebsiella* sp., *Staphylococcus* sp., *Salmonella* sp. and *Bacillus* sp. (turbidity compared with the McFarland standard) were prepared and lawn on to MHA agar after that 100 μ l of the hot water extract, ethanol- and methanol extracts at a concentration of ~11.1mg/ml each were introduced into the wells (8 mm³). Absolute ethanol and methanol and water were used as negative controls while the gentamicin (10 μ l) was used as positive control. Plates were incubated at 37°C for 12-18 hours and examined for formation of the zone of inhibitions (mm).

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Determination of Minimal Inhibitory Concentration (MIC)

The minimal inhibitory concentration (MIC) of the betel leaves was demonstrated through multiple tube dilution method^{2,20}. An aliquot of 100 µL of each bacterial culture (turbidity adjusted with 0.5 McFarland standard) was inoculated into the appropriately labeled sterile tubes containing Mueller Hinton (MH) broth (Oxoid Ltd, England). Afterward the 10g of different betel leaves powder were aseptically mixed with 90ml of distilled water. Different concentration of leaf extracts such as 16 µL, 32 µL, 64 µL, 128 µL, 256 µL, 512 µL, 1024 µL and 2048 µL were introduced into the bacterial culture containing Mueller Hinton broth² microtubes. After incubation at 37 °C for 24 hours all the tubes were observed and recorded the lowest concentration (mg/mL) of each sample in which the bacterial cell was found to grow and considered as the MIC value².

Results and Discussion

Most of the people of the developing countries are dependable on different herbal therapy, homeopathy, Ayurveda, Unani, and home remedies due to the minor side effects and deficient cost^{2,21,22}. As people are more dependent on herbal medicine than the synthetic medicine, it is essential to ensure the extrinsic and intrinsic quality of the natural ingredients of herbal medicine during cultivation, processing and formulation to ensure safety of public health.

Assessment in vitro antibacterial activity of the betel leaf through extraction methods

Current investigation showed that ethanol and methanol extract of all samples exhibited their antimicrobial properties against the tested bacteria.

The ethanol extracts of sweet betel leaf was found to form zone of inhibition against *Pseudomonas* sp. (15mm) and *Listeria* sp. (12mm), respectively while the methanol extract showed zone of inhibition in 15mm in diameter against only the *Listeria* sp. (15mm) (Table 1).

In case of ethanol extract of hot betel leaf, the maximum activity was found in *E. coli* (18mm) and *Vibrio* sp. (16mm). The sample showed their potency against almost all tested bacteria except *Klebsiella* sp. and *Staphylococcus* sp. On the other hand the methanol extracts of the samples were found to be effective against all the bacteria. The clear zone of diameter was observed against *E. coli* (20mm), *Pseudomonas* sp. (18mm) *Vibrio* sp. (14mm) *Bacillus* sp. (14mm) *Klebsiella* sp. (14mm) *Staphylococcus* sp. (15mm) *Listeria* sp. (13mm) and *Salmonella* sp. (15mm) consecutively (Table 1). Surprisingly, both ethanol and methanol extracts of the samples were tremendously reduced the growth of *E. coli* more effectively than of gentamycin used as positive control.

The ethanol and methanol extracts of fresh betel leaf was found to be effective against *Vibrio* sp., *Klebsiella* sp., *Listeria* sp. and *Salmonella* sp. In case of ethanol extract a clear zone was observed against *Vibrio* sp. (12mm), *Klebsiella* sp. (11mm), *Listeria* sp. (15mm) and *Salmonella* sp. (31mm), likewise methanol extract of the samples showed their bactericidal activity against *Vibrio* sp. (13mm), *Klebsiella* sp. (13mm), *Listeria* sp. (11mm) and *Salmonella* sp. (9mm) (Table 1).

According to our findings, the in-vitro antimicrobial activities of the betel leaves extracts, especially ethanol and methanol extracts were prominent against microorganisms. Also, interestingly the both ethanol and methanol extracts of all the samples significantly exhibited antimicrobial activity against *Listeria* sp.

Detection of MIC values of the betel leaf through broth micro-dilution methods

In the current study the lowest MIC value of sweet betel leaf extract was recorded against *Pseudomonas* sp. (50 mg/ml), whereas, the highest MIC values of hot betel leaf and fresh betel leaf extracts was recorded 1 mg/ml against *E. coli* and *Klebsiella* sp., respectively (Table 2).

The experiments were conducted three times independently, and the results were found to be reproducible. One representative data has been shown.

Table 1. Antibacterial activity of ethanol and methanol extracts of betel leaves

Test organisms	Zone of inhibition in diameter (mm)						Gentamycin (10 µg)
	Sweet betel leaf		Hot betel leaf		Fresh betel leaf		
	Ethanol	Methanol	Ethanol	Methanol	Ethanol	Methanol	
<i>E. coli</i>	-	-	18.0	20.0	-	-	16.8
<i>Pseudomonas</i> sp.	15.0	-	10.0	18.0	-	-	28.4
<i>Vibrio</i> sp.	-	-	16.0	14.0	12.0	13.0	18.0
<i>Bacillus</i> sp.	-	-	10.0	14.0	-	-	22.0
<i>Klebsiella</i> sp.	-	-	-	14.0	11.0	13.0	18.8
<i>Staphylococcus</i> sp.	-	-	-	15.0	-	-	22.0
<i>Listeria</i> sp.	12.0	15.0	12.0	13.0	15.0	11.0	23.0
<i>Salmonella</i> sp.	-	-	15.0	15.0	31.0	9.0	29.2

Table 2. Minimum Inhibitory Concentration (MIC) of the samples studied

Samples	MIC(mg/ml)							
	Test organisms							
	<i>E. coli</i> Sp.	<i>Klebsiella</i> Sp.	<i>Pseudomonas</i> Sp.	<i>Bacillus</i> Sp.	<i>Salmonella</i> Sp.	<i>Listeria</i>	<i>Staphylo-</i> <i>coccus</i> Sp.	<i>Vibrio</i> Sp.
Sweet betel leaf	25.0	25.0	50.0	25.0	25.0	25.0	25.0	25.0
Hot betel leaf	1.0	3.0	7.0	7.0	3.0	7.0	3.0	3.0
Fresh betel leaf	3.0	1.0	6.0	3.0	3.0	6.0	6.0	3.0

The MIC value of sweet betel leaf was found very prominent at 25 mg/ml against all the tested microorganisms only *Pseudomonas* sp. was found to be inhibited at 50 mg/ml. In case of hot betel leaf, *Klebsiella* sp., *Salmonella* spp., *Staphylococcus* sp., and *Vibrio* sp. found to be inhibited at 3 mg/ml, while the growth reduction of *Pseudomonas* sp., *Bacillus* sp. and *Listeria* sp. was observed at 7 mg/ml. Only for *E. coli* the MIC was recorded at 1 mg/ml. Likewise, the bioactive compound of fresh betel leaf was effectively reduced the growth of *E. coli*, *Bacillus* sp., *Salmonella* sp. and *Vibrio* sp. at 3 mg/ml, while the MIC was recorded at 6 mg/ml for *Pseudomonas* sp., *Listeria* sp. and *Staphylococcus* sp. Only the growth reduction of *Klebsiella* sp. was observed at 1 mg/ml. In our study all the samples were found to be effectively reduced the growth of 8 tested bacteria through broth dilution comparatively the ethanol and methanol extracts¹⁻².

Conclusion

Present investigations revealed the bacteriological profile of betel leaves as well as successfully chalk out the medicinal properties also which could significantly reduce the growth of pathogens. However, the plants or herbs as medicine may not retard the growth of pathogenic bacteria significantly, but this sort of findings would be helpful to formulate the combined drug with antibiotics.

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