The difference of antibacterial effect of neem leaves and stick extracts

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Purpose: This study determined the antibacterial effect of ethanolic neem leaves and stick extract in inhibiting the growth of *Sreptococcus mutans*.
Materials and Methods: Two different parts of neem, leaves and stick, extracts using ethanol were prepared at 10% and 20% (w/v: extraction powder/water) respectively. Each extractions were dropped on an MHA agar that had been inoculated with *Streptococcus mutans*. Distilled water was used as the control. After 24 hours of incubation, the inhibition diameters were measured. Collected data were analyzed by analysis of variance (ANOVA) followed by Least Significance Difference (LSD) at a 95% confinement level.
Results: The inhibition zone value of neem extracts on *Streptococcus mutans* showed that neem leaves extract had less inhibition value than neem stick extract on all concentrations. The ANOVA showed that there were significant influence of neem extracts ($p<0.001$), neem concentrations ($p<0.001$), and neem extract-concentration ($p<0.023$) on *Streptococcus mutans* inhibition.
Conclusion: Neem leaves and stick ethanolic extracts had antibacterial effect on *Streptococcus mutans*. Neem stick extract had higher antibacterial properties than the leaves extract. **(Int Chin J Dent 2007; 7: 27-29.)**
Key Words: antibacterial effect, neem extracts, *Streptococcus mutans*.

Introduction

The use of therapeutic plants has been beneficial to the oral health throughout the world for more than thousands years. Over centuries, different parts of neem plant, stem bark, root, leaves, seeds, etc., have been used in the Indian folk medicine. The advantage of traditional medicine is that it is less likely to form allergies and side effects. Widespread use of antibiotics in dental practice gives microorganisms enhanced opportunities for the development of resistance to a broad spectrum of antibiotics.[1] Neem is one of the most widely researched tropical trees as the source of therapeutic agents. The chemical compositions of neem extract have been analyzed since twenty years before. Many active components of neem, azadirachtin, salannin, meliantriol, or nimbin etc., have been identified, and the most active ingredient is reported as azadirachtin.[2] Clinical studies have shown that the neem leaves extract decreased the dental plaque index and *Streptococcus mutans* and *Lactobacilli* growth.[3,4] An in vitro study has demonstrated that aqueous extract from Neem leaves inhibits biofilm formation and adhesion in composite resin by *Candida albicans*.[5] Also it has demonstrated that formation of the bacterial plaque has been positively affected by aqueous neem stem bark extract.[6] Many experiments have tested the aqueous neem extract. On the other hand, the methanolic extract of neem has reported to have in vitro antiviral activity against group B coxsackieviruses[7] or against *Staphylococcus Aureus, Escherichia Coli, Pseudomanas Aeruginosa,* and *Candida albicans*.[8] The extraction methods might affect the antibacterial efficiency. However, it has not yet well examined the antibacterial effects of ethanolic extract of neem toward *Sreptococcus mutans* that has been recognized as the major organism involved in caries. Based on these backgrounds, this study was aimed to compare the influence of ethanolic neem leaves and stick extract in inhibiting the growth of *Sreptococcus mutans*.

Materials and Methods

Extract preparation

Neem leaves or sticks, 300 g, were selected, washed, cut into small pieces, and dried in an oven (45-50°C) for 3 days. Neem leaves or sticks were blended using a blender, then extracted using 96% ethanol for 10 hours. The extraction was carried out using soxhlet instrument as described previously.[8] Three doses of the dried extractions, 20 g of extract in 100 mL distilled water (20%), 10 g of extract in 100 mL distilled water (10%), and no extract in 100 mL distilled water (control) were prepared.

Bacterial sensitivity test

The *Streptococcus mutans* sensitivity test was carried out using the agar diffusion disc technique.[9] The *Streptococcus mutans* were cultured in the MHA agar for 24 hours at 37˚C. Five colonies were transferred into 2 mL of BHI. Its turbidity was compared to the Standard Brown III solution. The suspension (1 mL) was taken using a micropipette and inoculated on the MHA agar petri dish. A sterile spreader was used to spread the suspension evenly on the agar. Neem extracts (50 μL) at each concentration were dripped into the holes. The petri dish was incubated at 37˚C for 24 hours.

Inhibited zone measurement

The inhibited zone was measured as the area around the hole where no *Streptococcus mutans* was growing. The required area was measured from the edge of the hole to the outer border of bacterial inhibition. The diameter was measured using a sliding caliper with a precision of 0.01 mm. Each measurement was taken three times and the average of the three measurements for each zone was recorded.

Statistical analysis

Statistical analysis of the data was carried out using the analysis of variance (ANOVA). The ANOVA was then followed by post-hoc tests (Tukey-Kramer multiple comparison test).

Results

The average and standard deviations of the inhibited zones of Streptococcus mutans on MHA agar treated with neem extracts were shown on Table 1. The result showed that the antibacterial effects were significantly different among control, neem leaves, and stick extracts ($p<0.001$, ANOVA). Post-hoc test (Tukey-Kramer) showed that neem stick extract (at 20%) had significantly higher antibacterial property than the leaves extract ($p<0.01$). The neem stick extract had antibacterial properties as concentration dependent manner ($p<0.01$).

Table 1. The inhibition zone value of neem extract toward *Streptococcus mutans.*

Extract concentration (%)	Neem leaves Mean	SD	Neem stick Mean	SD	
0 (control)	0.000	0.000	0.000	0.000	NS
10	3.089 a	0.713	4.022 a, b	0.661	NS
20	3.857 a	0.408	5.778 a, b	0.211	p<0.01

SD, Standard deviation. NS: Not significant between the properties of leaves and stick extracts.
a, significantly different from control ($p<0.001$); b, significantly different between different concentrations ($p<0.01$).

Discussion

In this study, we have shown that the ethanolic neem stick extract had higher antibacterial properties than the leaves extract. Neem leaves had the active component of azadirachtin (1-3%), whereas the active component of neem stick is tannin (6%).[10] Both the azadirachtin and tannin belongs to the polyphenol group. The difference in the antibacterial effect was probably because of the different percentage in the active component of phenol groups in both neem leaves and sticks. Neem stick extract possessed a wide spectrum of antibacterial action against gram negative and gram positive microorganism.[11] Hydrolyzable tannins, gallotannins from crude drugs has an inhibitory activity against glucosyltransferase from *Streptococcus mutans*.[12] Gallotannin is hardly soluble in water but easily in ethanol. The reason that the neem stick extraction had higher antibacterial properties than the leaves extraction might be caused by the extraction solvent in this study differ from previous studies.[5,6] Table 2 also showed that both extract and concentration of neem influence the inhibition zone value. The neem leaves extract had less inhibition zone value comparing to the neem stick extract on the concentration of 10 and 20% by ethanol extraction method. By this finding, it seems necessary to study further about the characteristic of the active components and their extraction methods which affect the antibacterial properties.

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