

## ANTIBACTERIAL EFFECTIVENESS OF NONI EXTRACT (*MORINDA CITRIFOLIA* L.) ON THE GROWTH OF *FUSOBACTERIUM NUCLEATUM* (ATCC® 25586™) AS AN ALTERNATIVE MATERIAL FOR ROOT CANAL IRRIGATION- AN IN-VITRO STUDY

Trelia Boel<sup>1</sup>, Dennis<sup>2</sup>

<sup>1</sup>Faculty of Dentistry, Department of Dentomaxillofacial Radiology, Universitas Sumatera, Utara, Medan, Indonesia.

<sup>2</sup>Faculty of Dentistry, Department of Conservative Dentistry, Universitas Sumatera, Utara, Medan, Indonesia.

### ABSTRACT

#### BACKGROUND

Irrigation solution is needed to lower the number of microorganisms in the root canal. Irrigation solution commonly used have flaws. It required the development of alternative irrigation materials from natural ingredients, one of which is the fruit of noni because it has antibacterial properties and is a biocompatible bonding agent. This research aims to know the antibacterial effect of the ethanol extract of the fruit of noni on *Fusobacterium nucleatum* by determining the value of the Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC).

#### METHODS

The research starts with drying noni fruit (3 kg) and obtained 400-gram of simplisia extracted with 5 L of ethanol 70% solvent, then evaporated to 50 grams. Antibacterial test was done using the method of dilution and diffusion. Dilution method-determination of turbidity was done by observing the MIC tubes visually and compared with control McFarland after being incubated for 24 hours at a temperature of 37°C, then amount of bacteria was calculated to get a value of MBC. Diffusion methods-using paper discs that absorb extract. Drag zone formed around the disk is measured with a ruler. Data obtained from this study is analysed with the test of Kruskal-Wallis and Mann-Whitney.

#### RESULTS

In dilution method, the tube starts to look crystal clear at a concentration of 12.5% and the absence of bacteria at a concentration of 25% to 100%. In diffusion method, the smallest zone was seen at concentrations of 12.5%.

#### CONCLUSIONS

Ethanol extract of noni fruit has antibacterial effects against *Fusobacterium nucleatum* MIC 12.5% to the value and the value of the MBC 25%.

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#### BACKGROUND

Microorganisms are the main aetiology of pulpal and periapical diseases. The decline in the number of microorganisms in the root canal is the main goal of treatment of endodonti.<sup>1</sup> Mechanical procedure alone is not sufficient to lower the amount of microorganisms and clean the root canal in total. Therefore, irrigation solution should be used in order to rinse the entire root canal system.<sup>2</sup> If irrigation is not done, then the smear layer produced during the preparation cannot be eliminated so that bacteria will remain inside the root canal. One of the bacteria that is the species most commonly isolated from infection of *Fusobacterium nucleatum* is root canal. *Fusobacterium nucleatum* is an obligate anaerobic gram-negative bacterium contained in the oral cavity.<sup>3</sup> *Fusobacterium nucleatum* and its products is the cause of the abnormalities pulpa and developments the occurrence of periapical lesions.

Guimarães et al. (2011) shows that of the 20 root canals pulp necrosis there was bacteria *Fusobacterium nucleatum* as much as 68.5%.<sup>4</sup>

Irrigation solution is necessary in root canal chemo-mechanical preparation.<sup>2</sup> Root canal irrigation solution used is Sodium Hypochlorite (NaOCl), Chlorhexidine (CHX), Ethylenediamine Tetra-Acetic Acid (EDTA).<sup>5</sup> Due to the weakness of some materials in irrigation eliminate bacteria and an increase in bacterial resistance against antimicrobial as well as side effects inflicted, this encourages researchers to locate herbal alternatives.<sup>6</sup> According to the WHO (2003), medicinal herbs have been widely accepted throughout the country. As much as 80% of the population in Africa using herbal remedies for the treatment of primary. The use of traditional herbal remedies is safer because it has relatively few side effects compared to synthetic drugs, it is easier to obtain, and the price is cheaper. One is fruit of noni (*Morinda citrifolia* L.).

Noni (*Morinda citrifolia* L.), also known by the fruit of "Mengkudu". Noni has a variety of pharmacological properties including the effect of antibacterial, analgesic, a bitter taste, antifungal, antiviral, and antioxidants. Noni fruit are also biocompatible bonding agent and does not cause injury against soft tissue as well as non-toxic.<sup>7</sup> Some literature indicates that noni fruit has antimicrobial effects and therapies as well as potentially to be used as endodontic irrigant. Some properties of the noni fruit such as the effect of antibacterial and can eliminate smear layer on the

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*Corresponding Author:*

Trelia Boel,

SL Alumni, No. 2,

Medan 20155, Kampus USU,

Medan,

Indonesia.

E-mail: trelia.boel@gmail.com

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concentration of 6%, enabling the use of noni fruit as a root canal irrigation materials.<sup>8</sup> From the results of research on micronutrient and phytochemicals from fruit screening whether the ingredient was saponins, tannins, alkaloids, flavonoids, phenols, anthraquinone, and terpenoids.<sup>9</sup>

Some research on the antibacterial effect of the extract of noni fruit has also been done with testing on other bacteria. Research Prabhakar, Priyanka, Basappa (2013) reported that the results of whether fruit extract concentrations that can fight bacteria *Enterococcus faecalis* is 6%.<sup>10</sup> Research results Kumarasamy et al. (2006) showed results, 0.1% noni fruit extract effectively inhibits the growth of *Streptococcus mutans* ( $19 \pm 0.5$  mm) and *Streptococcus mythic* ( $18.6 \pm 0.3$  mm). Minimum concentration extracts of noni that can inhibit the growth of *Streptococcus mutans* is 0, 0125% and *Streptococcus mitis* is 0, 00625%.<sup>11</sup> Candida et al. (2001) reported that the minimum concentration of ethanol extract of noni can inhibit the growth of bacteria *Staphylococcus aureus* is at a concentration of 0.1%.<sup>12</sup>

Study that has been conducted in Indonesia regarding the development of natural materials as an alternative to root canal treatment against bacteria *Fusobacterium nucleatum* such as study by Leonardi (2015) the ethanol extract of the tuber radish (*Raphanus sativus* L.) against *Fusobacterium nucleatum* MIC results obtained at a concentration of 12.5% and MBC at a concentration of 25%.<sup>13</sup>

Based on the ability of noni extract for its antibacterial properties and can eliminate the smear layer, then carried out research on the antibacterial effect of the ethanol extract of noni (*Morinda citrifolia* L.) against *Fusobacterium nucleatum* (ATCC 25586<sup>®</sup>™) as an alternative to root canal irrigation. In this study, researchers will use the concentration starting from 100% of Noni extract to investigate its value of MIC and MBC.

## METHODS

This design of study is in-vitro study. The type of research in the form of experimental research with design laboratory research post-test only control group design. The samples used in the study of bacterial colony of *Fusobacterium nucleatum* is (ATCC<sup>®</sup> 25586™) that have been isolated and were bred with the Soy Agar medium Trypticase (TSA).

Making the noni fruit extract conducted by maceration technique using ethanol 70% and dilution condensed thick extract of noni to get a concentration of extract 100%, 50%, 25%, 12.5%, 6.25%, 1.56%, and 3, 125%.

Testing the effectiveness of conducted in dilution (dilution) using the medium Trypticase Soy Broth (TSB). At each concentration of *Fusobacterium nucleatum* was then added a suspension as standard solution of 0.5 Mc Farland, Vortex was performed until homogeneous and incubated in a CO<sub>2</sub> incubator at 37°C for 24 hours, and was observed whether precipitation or not. The tube with the lowest concentrations are not formed precipitation show the effect bacteriostatic and the concentration is called MIC.

All the tubes are not formed precipitate then do subcultures on media Trypticase Soy Agar media (TSA) and incubated in a CO<sub>2</sub> incubator at 37°C for 24 hours. The media TSA on a petri dish with the lowest concentrations are not formed colony growth shows the effect of the concentration of bacteriocidal and is called MBC.

Each concentration done repetition as much as four times to prevent the occurrence of refraction and views the median concentrations of MIC and MBC ethanol extract of noni.

## RESULTS

MIC concentration obtained with the method of dilution and diffusion. MIC dilution methods, observing each concentration samples starts to look clear when compared to the control of Mc Farland. On observation, concentration starts to look crystal clear is 12.5%. MIC diffusion methods, observing the smallest concentration which still produces inhibitory zones. On observation, the smallest concentration that produced inhibitory zone is 12.5%.

MBC concentration obtained with the method the Pour Plate. Results from the fourth repetition, by looking at the smallest concentration samples of media Trypticase Soy Agar that there is no bacterial growth or sterile. The observations of each repetition, the smallest concentration of a sterile is 25%.

| Effectiveness | N | Med    |
|---------------|---|--------|
| MIC           | 4 | 12, 5% |
| MBC           | 4 | 25%    |

**Table 1. MIC Concentration and Ethanol Extract of Noni Fruit MBC Against *Fusobacterium Nucleatum* Growth**

Results of the four repetition of noni extract of *Fusobacterium nucleatum* towards growth, then conducted data analysis to get the median or middle value concentration MIC and MBC. In table 1, was obtained that the concentration of the MIC is 12.0% and the concentration of the MBC is 25%. No statistical analysis was applied as there were only 4 repetition for each group.

## DISCUSSION

The results of this study demonstrate the effectiveness of noni fruit ethanol extracts with solvents against *Fusobacterium nucleatum* growth. MIC concentration of 12.5% and the concentration of MBC by 25%.

As for the factors that affect the ability of the noni extract so are bacteriostasis and bacteriocidal i.e. contain active compounds such as tannins, flavonoids, saponins, alkaloids, and anthraquinone. Saponin is active substances that have properties such as soap that can dissolve dirt. Working mechanism namely antibacterials saponins can cause leakage of proteins and enzymes of the cell.<sup>14</sup> Tannins in low concentrations is capable of inhibiting the growth of bacteria, while at high concentrations, tannins works as antimicrobial with how bacteria protoplast coagulation, so a stable bond formed with protein of bacteria.<sup>15</sup>

Flavonoid compound phenol that have a tendency to bind to proteins, so disrupting the process of metabolism. Compounds of flavonoids may damage the cytoplasmic membrane, which causes leaking of important metabolites and disabling bacterial enzyme systems. This damage allows the nucleotides and amino acids on bacteria seeped out and prevent the inclusion of the active substances into the cell and can cause cell death.<sup>16</sup>

The alkaloid is capable of inhibiting cell wall synthesis which will cause lysis cell so that the cell dies. The mechanism is alleged by way of disrupting the Peptidoglycan constituent components on a bacterial cell, so that the layer of

the cell wall is not perfectly formed. And will cause cell lysis either prone to physical or osmotic and cause cell death.<sup>17</sup>

### CONCLUSIONS

Ethanol extract of fruit noni (*Morinda citrifolia* L.) can inhibit the growth of *Fusobacterium nucleatum* (ATCC® 25586™) in vitro with a value of the MIC of 12, 5% and the value of the MBC of 25%.

### REFERENCES

- [1] Gajan EB, Aghazadeh M, Abashov R, et al. Microbial flora of root canal of pulpally-infected teeth: *Enterococcus faecalis* a prevalent species. *J Dent Res Dent Clin Dent Pros* 2009;3(1):24-7.
- [2] Carrotte P. Endodontics: Part 7. Preparing the root canal. *Br Dent J* 2004;197(10):603-13.
- [3] Nwaokorie et al. AP-PCR and antimicrobial susceptibility patterns of *Fusobacterium nucleatum* associated with chronic periodontitis among patients at Lagos University Teaching Hospital. *Br Microbiol Res J* 2012;2(2):97-8.
- [4] Guimaraes NLS, Otoch HM, De Andrade LC, et al. Microbiological evaluation of infected root canals and their correlation with pain. *RSBO* 2012;9(1):31-7.
- [5] Peters OA, Peters CI. Cleaning and shaping of the root canal system. In: Cohen S, Hargreaves KM, eds. *Cohen's Pathways of the pulp*. 9<sup>th</sup> edn. Canada: Elsevier Mosby 2006: p. 318-22.
- [6] Garg P, Tyagi SP, Sinha DJ, et al. Comparison of antimicrobial efficacy of propolis, *Morinda citrifolia*, *Azadirachta indica*, *Triphala*, green tea polyphenols and 5.25% sodium hypochlorite against *Enterococcus faecalis* biofilm. *Saudi Endodontic J* 2014;4(3):122-7.
- [7] Pujar M, Makandar S. Herbal usage in endodontics - a review. *IJCD* 2011;2(1):34-7.
- [8] Sari L. Utilization of traditional medicine with the benefits of security considerations. *Kefarmasian Science Magazine* 2006;3(1):1-2.
- [9] Saghiri MA, Garcia-Godoy F, Asgar K, et al. The effect of *Morinda citrifolia* juice as an endodontic irrigant on smear layer and microhardness of root canal dentin. *Oral Sci Int* 2013;10(2):53-7.
- [10] Anugweje KC. Micronutrient and phytochemical screening of a commercial *Morinda citrifolia* juice and a popular blackcurrant fruit juice commonly used by Athletes in Nigeria. *World Rural Observations* 2015;7(1):40-5.
- [11] Prabhakar AR, Basavraj P, Basappa N. Comparative evaluation of *Morinda citrifolia* with chlorhexidine as antimicrobial endodontic irrigants and their effect on micro-hardness of root canal dentin: an in vitro study. *Int J Oral Health Sci* 2013;3(1):5-9.
- [12] Kumarasamy B, Manipal S, Duraisamy P, et al. Role of aqueous extract of *Morinda citrifolia* (Indian Noni) ripe fruits in inhibiting dental caries-causing *Streptococcus mutans* and *Streptococcus mitis*. *J Dent (Tehran, Iran)* 2014;11(6):703-10.
- [13] Candida T, Franca JP, Chaves AL, et al. Evaluation of antitumoral and antimicrobial activity of *Morinda citrifolia* L. Grown in Southeast Brazil. *Acta Cirurgica Brasileira* 2014;29(Suppl 2):10-4.
- [14] Leonardi A. Antibacterial effect of the ethanol extract of tuber of radish (*Raphanus sativus* L.) against *Fusobacterium nucleatum* ATCC 25586 as alternative medikamen root canal (in-vitro fertilization). *Terrain: Thesis USU*, 2015.
- [15] Thakur M, Melzig MF, Fuchs H, et al. Chemistry and pharmacology of saponins: special focus on cytotoxic properties. *Botanics: Targets and Therapy*, Dove Medical Press Ltd., 2011;1:19-29.
- [16] Levison ME. Pharmacodynamic of antimicrobial drugs. *Infect Dis Clin North Am* 2004;18(3):451-65, vii.
- [17] Omojate GC, Enwa FO, Jewo AO, et al. Mechanisms of antimicrobial actions of phytochemical against enteric pathogens- a review. *J Pharm Chem Biol Sci* 2014;2(2):77-85.