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Effectiveness of *Lawsonia inermis* L. Leaves Methanol Extracts on Gingivitis Healing (*In vivo* Study on *Sprague dawley* Rats)

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Authors' contributions

This work was carried out in collaboration between both authors. Author ZL managed the literature searches, designed the study and the experimental process. Author SJ analyses the study performed, wrote the protocol and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Lawsonia inermis L. leaves has been used to healing wound and antibacterial caused of active content like essential oils, steroids, triterpenes, saponins, flavonoids and tannins. *Lawsonia inermis* L. leaves methanol extract showed effective against *S. sanguinis* with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) at 31.250 µg/mL and no toxic as tested in mice and human gingival fibroblasts. This study aims to examine the effectiveness of *L. inermis* L. leaves methanol extracts to heal gingivitis in *Sprague dawley* rats. Forty subjects were divided into group A (n=30) and B (n=10). A group created artificial inflammation in the mandibular labial gingiva with 10% H_2O_2. Group A was divided into 3 groups of treatment, positive, and negative control groups. Treatment groups were given *Lawsonia inermis* L. leaves methanol extract in 3 concentrations (62.500, 31.250, and 15.625 µg/mL). Positive and negative control groups were given povidone Iodine 1% and aquabidest respectively. Group B as an healthy rats group was divided into 15.625 µg/mL concentration and aquabidest groups. Histopathological

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changes were observed on day 3 by the condition of gingival epithelium, epithelial connective tissue relationships, and the distribution of inflammatory cells. Statistic analysis showed no difference in healing between the three concentrations of *Lawsonia inermis* L. leaves methanol extract and *povidone iodine* (α=0.694>0.05) while there were differences among the 3 concentrations. Higher concentration (62.500 µg/mL) can accelerate the inflammatory cells reduction and epithelial connective tissue relationships repair. It was concluded that *Lawsonia inermis* L. leaves methanol extract can heal gingivitis at concentration up to 62.500 µg/mL.

Keywords: Lawsonia inermis L. leaves methanol extract; gingivitis; healing process; inflammatory cells; epithelial connective tissue relationships.

1. INTRODUCTION

In general, 39 % of Indonesian population have oral and dental health problems. In East Indonesian villagers, its prevalence on 25 – 64 years old group is higher than those of national prevalency [1]. Gingivitis is the most common oral inflammatory condition found in developing countries that is in chronic condition. Inflammation process of chronic gingivitis may extend into deeper periodontal apparatus resulted in periodontitis in form of destruction of attachment apparatus lead to the mobility and loss of teeth [2].

Inflammation of periodontal tissue is caused of variety causative agents included bacteria and trauma. Accumulation of microorganism within dental plaque acts as a main etiological factor. Another factor may trigger periodontal disease included calculus, poor oral hygiene, crowding, caries, bad restoration [3].

Inflammation process started on epithelial surface and part of connective tissue. Hemorrhage will be covered immediately by fibrin and blood clott. In hours, oedem occured and neutrophile increased. Neutrophile started to migrate on decay surface and finally there is crustae formation on the surface epithelium. Crustae, neutrophile and blood clott appear within 24 hours. Crustae consisted of fibrin, blood red cells, and dead neutrophile. Clinically, crustae appears as necrotic area with white or yellowish color. This protects the underlying epithelial and connective tissue until epithelium recovering this area [4]. At the same time, there is cellular activity within connective tissue. Macrophage appeared and together with neutrophile started to phagocyte necrotic cells and debris. On day one, fibroblast and capillary vessels started to proliferate, epithelial cells on the edge of surface started migrating to opposite side.

Epithelial cells extended into area between crustae and underlying tissue. As soon as the migration and regeneration of epithelial cells, connective tissue and skeletal muscle regenerated within the connective tissue. On day 2 to 7, mitosis, granulation tissue, macrophage, fibroblast, and new capillary vessels occured On day two, granulation tissue appear more distinct, new capillary vessels covered the wound extensively. Tissue looks more redness and shiny around the ulcer. Granulation tissue consisted of chronic inflammatory cells, vascular supply for nourishing in order to form a new connective tissue [4]. On several days, when epithelial cells of each side meet, the migration stop and crustae disappear. Collagen started to be remodelling and rearrangement. Collagenases enzyme remove the excessive collagen. Lack of collagenases resulted in excessive connective tissue formation.

If the tisue destruction is in light condition and occured in the short time, fibrosis is not found markedly, especially on oral mucosa. At this condition, regeneration of connective tissue, muscle and nerve fibre easily occured. Inflammatory cells and vascular supply decrease and tissue recovered. Fibrous tissue, wound contraction, epithelisation occured within several weeks. The wound will be closed by contraction of connection tissue beside the migration of epithelial [4].

The main purpose of therapy of gingivitis is to eliminate the etiological factor in order to diminish or eliminate inflammation lead to healthy gingiva. The professional treatment and maintenance of periodontal tissue are important to be fullfiled to prevent the occurence of inflammation. The therapy of gingivitis include removal the causative factors such as dental plaque and calculus, maintenance of oral hygiene [2].

Dental plaque can be removed by tooth brushing and interdental cleaner. Antibacterial compound

is often needed to help removing inflammation by inhibiting the bacteria growth and decreasing its concentration within dental plaque [5]. Application of antimicrobial agent on gingivitis has been proven effective in decreasing the depth of pocket and periodontal bacteriae pathogen number, moreover to obtain optimal treatment [6].

Lawsonia inermis Linnaeus has synonim name as *Lawsonia spinosa* L., *Lawsonia alba Lamk* (LANK), or *Lawsonia ruba* [7]. In United Kingdom, *Lawsonia inermis* L. is popular with the name of '*henna'* or '*camphire'*. In Indonesia often called as 'inai'.

Lawsonia inermis L is a much-branched glabrous shrub or small tree 2-6 m in height, which may be spiny, bark greyish-brown, unarmed when young, older plants with spine-tipped branchlets. Young branches quadrangular, green but turn red with age. Its small dark green leaves smells good as rose flower (Figs. 1 and 2). This pale sulphur like or light pink color flower smells good and give red brownish color in night which is called as '*henna*' [7]. *Lawsonia inermis* L. originated from east of Africa, north of Asia, and Australia. Also found in tropic areas include America, Mesir, India, and a part of Mediteranean. *L. inermis* L. first grows at tropical prairie and dry area extended from Africa to the circle of west Pacific. This tree is often found in dry forest with climate changing, most of them seen as fence plantation [9].

In Indonesia, 'inai' commonly used in certain Indonesian villagers to treat skin wound. Study used topical application of *L. inermis* L. leaves ethanol extract, 200 mg/kg body weight/day showed significant effect of increasing wound contraction, epithelisation, and granulation tissue formation on incision and excision skin wound rats. After ten days, there was increasing of collagen fibers arrangement, fibroblast and decreasing of inflammatroy cells [10].

Lawsonia inermis L leaves contain antibacterial compounds needed in healing process [8]. *Lawsonia inermis* L. leaves extract contain active antibacterial compounds include essential oil (+), steroid (+), triterpen (+), saponin (++), flavonoid (+++), and tannin (++++). 11 *Lawsonia inermis* L. leaves infusion is effective against *Aggregatibacter actinomycetemcomitans* [4] *Lawsonia inermis* L. leaves extract has MIC (*Minimum Inhibitory Concentration*) and MBC (*Minimum Bactericidal Concentration*) against *Streptococcus sanguinis* ATCC 10556, at the concentration of 31.250 µg/mL. *Lawsonia inermis* L. leaves extract also do not show cytotoxicity on human gingival fibroblast cell at the concentration from 10.000 to 640.000 µg/mL [11].

Following the previous research of *L. inermis* L. leaves and based on those facts above, the temporary assumption is *L. inermis* L. leaves can be used to treat oral mucosa wound include gingivitis, therefore this research was conducted to find out the effect of *L. inermis* L. leaves methanol extract (LiLLME) on the healing of induced gingivitis in *Sprague Dawley* rats subject. If this herb substances give good result, it will be continued on human research to find out the new herbal oral rinse product which is effective to reduce dental plaque and able to cooperate with another instrument therapy of periodontal disease especially gingivitis.

Fig. 1. Plant *L. inermis* L. [7]

Fig. 2. *L. inermis* L. tree in batam island Indonesia [8]

2. MATERIALS AND METHODS

A total of 40 sample with the age of 3-4 months and weighing of 150-200g from Biomedical and Pharmacy Centre of Research and Developmental Health Division, Republic of Indonesia used in this experimental *in vivo* laboratory study. All of subjects were treated by health standard condition and separated between male and female.

Lawsonia inermis L. Leaves extract used in this study was made of maceration technique used metanol 70%. Dried simplisia of *L. inermis* L. leaves was ground and as much as tenth part filled in macerator, poured with seventy fifth part of methanol solution without any exposure of sunlight for 5 days and mixed it occasionally. Then, filtered the simplisia and added the sediment with solution to 100 part. The liquid and sediment were filled in closed basin for 2 days at cool room temperature without any exposure of sunlight. The filtration was evaporated by light pressure rotary evaporator at the temperature of <50°C, resulted in dry extracts.

The concentrations of 1/16, 1/32, and 1/64 which equal to 62.500, 31.250, and 15.625 µg/mL respectively were made by diluting 10 g LiLLME in aquabidest. To find out several concentrations of extract, the serial dilution method used in this study, as followed: as much as 10 g (10.000 mg) of LiLLME was diluted within 10 mL aquabidest, resulted in 10 mL of 1.000 mg/mL LiLLME solution. As much as 10 mL of 1.000 mg/mL LiLLME solution diluted with 10 mL aquabidest resulted in 20 mL of 500 mg/mL LiLLME solution. As much as 20 mL of 500 mg/mL LiLLME solution diluted with 20 mL aquabidest to get 40 mL of 250 mg/mL LiLLME solution. As much as 40 mL of 250 mg/mL LiLLME solution diluted with 40 ml aquabidest to get 80 mL of 125 mg/mL LiLLME solution. The same process was done up to getting 160 mL of 15.625 µg/mL LiLLME solution. Finally, there were 80 mL of 62.500 µg/mL, 80 mL of 31.250 µg/mL, and 160 mL of 15.625 µg/mL LiLLME solution provided.

The research subjects (N=40) was divided into 2 groups, A and B. Group A (n=30) was rubbed on their labial mandibular gingiva with H_2O_2 10% to conduct induced gingival inflammation or gingivitis while group B (n=10) as healthy control group. Group A divided into 3 sub groups: treatment (n=18), positive control (n=6) and negative control (n=6) group. Treatment gingivitis group treated with topical application of 3 concentrations @ 6 subjects (62.500, 31.250, and 15.625 µg/mL LiLLME). Control positive (n=6) and negative (n=6) gingivitis group treated with *povidone iodine* 1 % and aquabidest topical application respectively. Group B as healthy group divided into 15.625 µg/mL LiLLME (n=5) and aquabidest (n=5) groups. Cotton buds used for topical application of 1 to 1,5 mL LiLLME, three times daily @ 5 minutes, interval 90 minutes for 3 days, as well as those of *povidone iodine* 1% and aquabidest.

On day 3, all subjects were executed under ether and the speciments derived from labial mandibular gingiva fixed in formaldehid 10%. The section was 3 µm. Histopathological features were evaluated under 10x and 20x objective magnification to observe the gingival epithelium (normal, mild, severe hyperplasia, eroded or ulcerated), epithelial underlying connective tissue junction (normal, detach) and the intensity of inflammatory cells (normal/mild, moderate, high).

3. RESULTS

Clinical evaluation on day 3 as followed: on 62.50 µg/mL gingivitis treatment group, gingiva look light pink, slightly swelling; on 31.25 µg/mL, gingiva look slightly red and swelling with epithelial ulceration; on 15.625 mg/mL, gingiva look red and slightly swelling (Fig. 3).

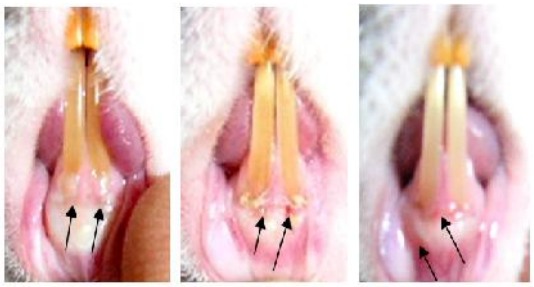

Fig. 3. Day 3: A. 62.500 µg/mL gingivitis group: Gingiva look light pink, slightly swelling, B. 31.250 µg/mL gingivitis group: gingiva look slightly red and swelling, ulcertion noted, C. 15.625 µg/mL gingivitis group: gingiva looks red and slightly swelling

On day 3, the histopathological features of gingivitis group (62.50 µg/mL), epithelial and its junction with underlying connective tissue look normal with mild inflammatory cells. At gingivitis group (31.25 µg/mL), epithelial looks like normal as well as its underlying connective tissue junction with moderate inflammatory cells. At

gingivitis group (15.625 µg/mL), epithelial has not in normal condition yet, its junction with underlying connective tissue look dettached with moderate inflammatory cells (Figs. 4, 5 and 6).

On day 3, the gingivitis groups of LiLLME showed new capillary vessels and collagen fibre formation within sub epithelial area (Figs. 7 and 8).

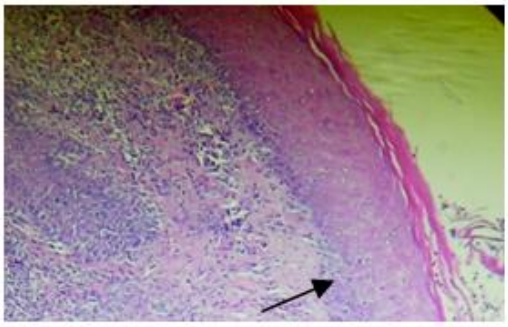

Fig. 4. Gingivitis group (62.500 µg/mL) showed normal epithelium as well as epithelial underlying connective tissue junction with mild inflammatory cells

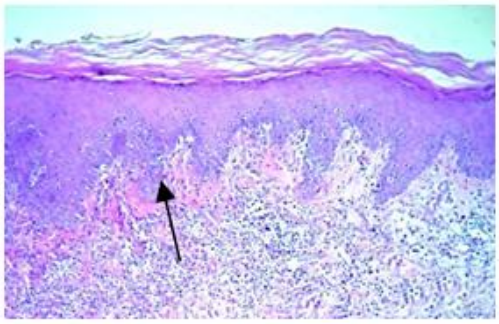

Fig. 5. Gingivitis group (31.250 µg/mL) showed epithelium almost in normal condition and normal epitheial connective tissue junction with moderate inflammatory cells

Data were analysed for those of gingival epithelium, epithelial connective tissue junction, and intensity of inflammatory cells using non parametric test of Kolmogorov – Smirnov, *Chi-Square*, and one way anova. The statitisctic analysis of gingival epithelium showed no statistical differences (α=0.694>0.05) between gingivitis treatment groups (concentrations of 62.500, 31.250, and 15.625 µg/mL LiLLME) with *povidone iodine* 1%.

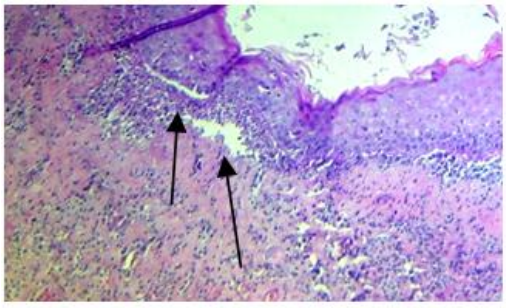

Fig. 6. Gingivitis group (15.625 µg/mL) showed epithelial not in normal condition, dettached epithelial connective tissue junction, and moderate inflammatory cells

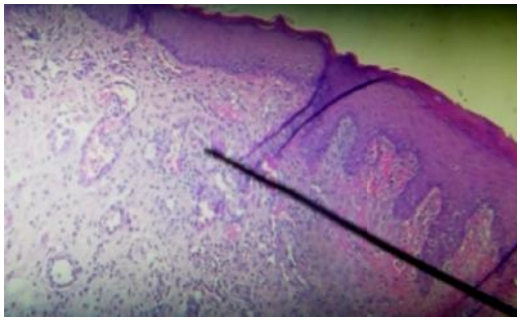

Fig. 7. New capillary vessels formation within sub epithelial area

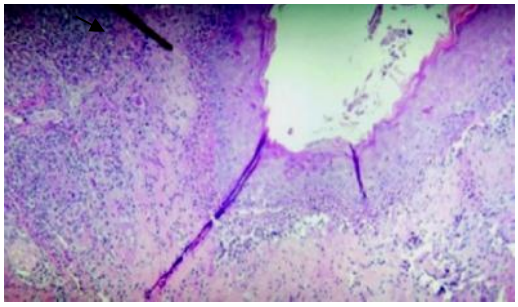

Fig. 8. Collagen fibre formation within sub-epithelial area

Table 1 showed the distribution of epithelial connective tissue junction condition. There was significant difference between epithelial connective tissue junction condition among gingivitis treatment groups. The higher concentration of LiLLME give the better healing (Fig. 9).

Table 2 showed the distribution of inflammatory cells intensity among subjects.

Table 1. The distribution of epithelial connective tissue junction condition

Group	Kinds of treatment	Epithelial connective tissue junction (%)		Healing (%)
		Normal	Dettach	
1	Treatment gingivitis group (**62.500** µg/mL LiLLME)	21.7	0	**100.0**
2	Treatment gingivitis group of 31.250 µg/mL LiLLME	17.4	9,1	65.6
3	Treatment gingivitis group of 15.625 µg/mL LiLLME	4.3	36,4	10.5
4	Positive controle gingivitis group (*povidone iodine* 1%)	8.7	27,3	24.2
5	Negative controle gingivitis group (aquabidest)	8,7	27,3	24.2
6	**Healthy controle group** of 15.625 µg/mL	21.7	0	**100.0**
7	**Healthy controle group** of aquabidest	17.4	0	**100.0**

Note: Percentage of healing process within epithelial connective tissue junction on gingivitis treatment group (62.500 µg/mL) is 100% as well as those of healthy controle group

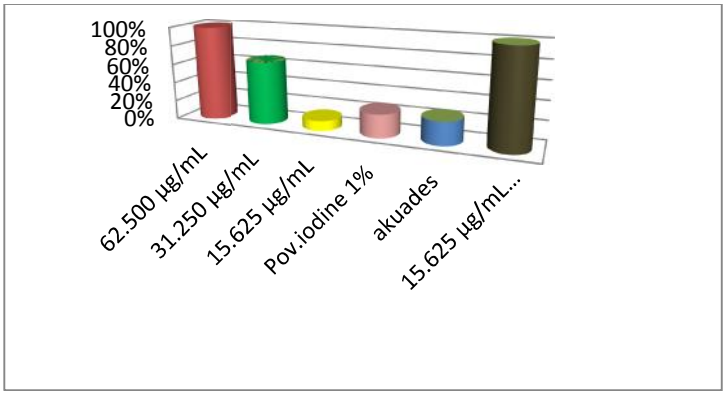

Fig. 9. Bar diagram of epithelial connective tissue junction healing process. The healing of this area reached 100% on treatment gingivitis group (62.500 µg/mL)

Table 2. The distribution of inflammatory cells intensity

Group	Kinds of treatment	Intensity of inflammatory cells (%)		
		None/Mild	Moderate	Severe
1	Treatment gingivitis group of **62.500** µg/mL LiLLME	**19,0**	15,4	0
2	Treatment gingivitis group of 31.250 µg/mL LiLLME	9,5	**23,1**	0
3	Treatment gingivitis group of **15.625** µg/mL LiLLME	4,8	23,1	**100**
4	Positive controle gingivitis group of *povidone iodine* 1%	9,5	23,1	0
5	Negative controle gingivitis group of aquabidest	14,3	15,4	0
6	Healthy controle group of 15.625 µg/mL	23,8	0	0
7	Healthy controle group of aquabidest	19,0	0	0

*Note: Among gingivitis group, the lowest inflammatory cells found mostly on treatment gingivitis group of **62.500** µg/mL LiLLME (19%) while the highest inflammatory cells on treatment gingivitis group of **15.625** µg/mL LiLLME (100%)*

4. DISCUSSION

From the clinical (inflammatory sign included color and swelling) and histopathological features (epithelial connective tissue junction and inflammatory cells intensity), the healing process showed less on 15.625 µg/mL group concentration among gingivitis group treated by LiLLME. One of the reason is the observation was on day 3 whereas the healing process is not complete yet. The study of Nayak et al. [10] revealed the significant effect of extract on skin wound healing process on day 7. Moreover, the healing process of induced gingivitis in this study is in the way of 'healing by second intention' which takes more time longer than those of 'healing by first intension' that occured in sulcular epithelium which commonly caused of bacterial invasion.

The gingival epithelium damage by bacteria is different with those of chemical toxic substances (H2O2 10 %) which applied in this study whereas chemical irritant resulted in more ulcerative inflammation in form of loss of epithelial surface caused of epithelial cells necrosis with expose underlying connective tissue or ulcer lesion [4]. Bacteria commonly attack sulcular epithelium of gingiva which is a non keratinized epithelium type that easier to be invaded by bacteria. The healing process of sulcular epithelium is ‘healing by first intention’ and different from those of ‘healing by second intention’ as occured in outer epithelium damage by chemical toxic irritant used in this study. Healing by second intention will take more time than those of primary intention healing [4].

The non significant differences of healing process between gingivitis group treated by 3 concentrations of LiLLME (62.5, 31.25 and 15.625 µg/mL) and those of *Povidone iodine* 1 % indicated that the effectivity of LiLLME on healing process equals to those of *Povidone iodine* 1 %. This due to active contents included essentials oil, flavonoids, saponins, steroid and triterpene, and tannins in LiLLME as anti-bacterial, anti-inflammatory, anti infection, anti-allergic, anti-mutagenic, anti-thrombosis, vasodilatory effect and phagocytes accelerator [12,13,14,15]. The use of essentials oil to help oral cleaning has been proven effective in decreasing of plaque and gingivitis therefore this often used to daily cleanser beside tooth brushing [16].

Essentials oil mouth rinse showed no significant effect with those of commercial product of *cetylpyridinium chloride* (CPC) mouth rinse against plaque and gingivitis [17]. These potential phytochemical components of LiLLME work through binding and destroyed effect of extracellular protein bacterial cell wall [13,18]. This fact was proven by the previous studies of Zubardiah (2006) about the potency of *Lawsonia inermis* L. leaves extract such as diffusion test that showed antibacterial effect of *L. inermis* L. leaves infusion against *Aggregatibacter actinomycetemcomitans* 4 and *Lawsonia inermis* L. leaves extract has MBC (*Minimum Bactericidal Concentration*) against *Streptococcus sanguinis* ATCC 10556 at concentration 31.250 µg/mL [11]. All of these bacteria have been known as pathogenic bacteria of gingivitis. The result of this study was also supported by the previous research by Zubardiah [11] that *Lawsonia inermis* L. leaves extract does not show any cytotoxicity effect on human gingival fibroblast up to concentration 640.000 µg/ml.

5. CONCLUSION

Healing process with topical application of 62.50 µg/mL *Lawsonia inermis* L. leaves methanol extract showed significant increase on day 3 whereas epithelial connective tissue junction has totally recovery therefore it was concluded that *Lawsonia inermis* L. leaves methanol extract able to heal gingivitis. This result is in accordance with clinical evaluation that *Lawsonia inermis* L. leaves methanol extract can decrease clinical sign of gingivitis in a concentration dependent manner started at the concentration 31.25 µg/mL.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Anonimous. Family folder health survey: community oppinion on status, coverage, response and health management system.

Research and development institution, Health Department Republic of Indonesia. 2004;3:18-20.
2. Novaes ABJr, de Souza SLS, Mário Taba Jr, Grisi MFdM, Suzigan LC, Tunes RS. Control of gingival inflammation in a teenager population using ultrasonic prophylaxis. Braz Dent J. 2004;1:15.
3. Hinrichs JE. The role of dental calculus and other predisposing factors. In: Newman MG, Takei HH, Klokkevold PR, Carranza FAJr, eds. Carranza's Clinical Periodontology. 10th ed. Saunders. St. Louis. 2006;170-92.
4. Kirkwood KL, Nisengard RJ, Haake SK, Miyasaki KT. Immunity and Inflammation: Basic Concepts. In: Newman MG, Takei HH, Klokkevold PR, Carranza FAJr, eds. Carranza's Clinical Periodontology. 10th ed. Saunders. St. Louis. 2006;209-27.
5. Perry DA. Plaque control for the periodontal patient. In: Newman MG, Takei HH, Klokkevold PR, Carranza FAJr, eds. Carranza's Clinical Periodontology. 10th ed. Saunders. St. Louis. 2006;728-48.
6. Perry DA, Schmid MO, Takei HH. Phase I Periodontal Therapy. In: Newman MG, Takei HH, Klokkevold PR, Carranza FAJr, eds. Carranza's Clinical Periodontology. 10th ed. Saunders. St. Louis. 2006;722-7.
7. Jones CC. Henna in the Ancient Egyptian Pharmacopoeia: The Ebers Papyrus. Kent State University; 2004. Available:http://www.hennapage.com/henna/mainindex.html. Accessed on September 30th, 2006
8. Zubardiah L. Antibacterial effect of *Lawsonia inermis* L. leaves extract on *Actinobacillus actinomycetemcomitans–in vitro*. Scientific Journal of Faculty of Dentistry. 2006;21(2):47-53.
9. Habbal OA, Al-Jabri AA, El-Hag AG. Antimicrobial properties of Lawsonia inermis: a review. Australian Journal of Medical Herbalism; 2007. Available:http://findarticles.com/p/articles/mi_6801/is_3_19/ai_n28486286 (Accessed on December 2nd, 2008)
10. Nayak BS, Isitor G, Davis EM, Pillai GK. The evidence based wound healing activity of *Lawsonia inermis* Linn. Phytother Res. 2007;21(9):827-31.
11. Zubardiah L. The effectivity of *Lawsonia inermis* L. leaves extract on gingivitis healing. Thesis of Doctoral Program on Faculty of Dentistry, University of Indonesia; 2009.
12. Raybaudi-Massilia RM, Mosqueda-Melgar J, Martin-Belloso O. Antimicrobial activity of essential oils on *Salmonella enteritidis*, *Escherichia coli*, and *Listeria innocua* in fruit juices. J Food Prot. 2006;69(7): 1579-86.
13. Miller AL. Antioxidant Flavonoids: Structure, Function and Clinical Usage. Available:http://www.thorne.com/altmedrev/fulltext/flavonoids1-2.html (Accessed on August 16th, 2006)
14. Davidson MW. Available:http://micro.magnet.fsu.edu/phytochemicals/pages/saponin.html. Accessed on August 16th, 2006.
15. Farouk AE, Ghouse FAH, Ridwan BH. New bacterial species isolated from Malaysian sea cucumbers with optimize secreted antibacteral activity. Am J Biochem Biotech. 2007;3(2):60-5.
16. Stoeken JE, Paraskevas S, van der Weijden GA. The long-term effect of a mouthrinse containing essential oils on dental plaque and gingivitis: A systematic review. J Periodontol. 2007;78(7):1218-28.
17. Albert-Kiszely A, Pjetursson BE, Salvi GE, Witt J, Hamilton A, Persson GR, Lang NP. Comparison of the effects of cetylpyridinium chloride with an essential oil mouth rinse on dental plaque and gingivitis - a six-month randomized controlled clinical trial. J Clin Periodontol. 2007;34(8):65-867.
18. Soegihardjo CJ. Flavonoid content and anti-hepatotoxic of *Sonchus oleraceus* L. Available:http://digilib.itb.ac.id/go.php?id=jbptitbpp-gdl-s3-1984-cjsoegihar-1735. (Accessed on August 16th, 2006)

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