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EFFECTIVENESS OF LAWSONIA INERMIS LINNEAUS LEAVES INFUSION IN GINGIVITIS HEALING

(EFEKTIFITAS INFUSA DAUN LAWSONIA INERMIS LINNEAUS TERHADAP PENYEMBUHAN GINGIVITIS)

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Abstract

Gingivitis is one type of periodontal disease and it is chronic in nature. The main etiological factor of gingivitis is bacterial plaque. Untreated gingivitis can spread to underlying tissues and it can become periodontitis and produce tissue destruction. One of the treatments for gingivitis is plaque removal, helped by antibacterial mouthrinsing to reduce bacterial plaque. *Lawsonia inermis* L. leaves have been used by Indonesian villagers for healing skin wounds and reported to show antibacterial effect in vitro and in vivo. This study aimed to determine the effect of *Lawsonia inermis* L. leaves infusion in gingivitis healing. A total of 63 gingivitis patients devided into 5 groups were instructed to rinse with 3 concentrations (50000 µg/mL, 10000 µg/mL, 5000 µg/mL) of *Lawsonia inermis* L. leaves infusion; 0.1% hexetidine solution, and placebo as control. Papilla Bleeding Index (PBI) used in measurement for bleeding on probing. The result showed that decreasing of PBI in *Lawsonia inermis* L. leaves infusion at 10000 µg/mL concentratation (80%), was stronger than hexetidine 0.1% (76%). In conclusion, *Lawsonia inermis* L. leaves infusion effective to reduce PBI better than hexetidine. In conclusion, *Lawsonia inermis* L. leaves is effective to treat gingivitis.

Key words: *Lawsonia inermis* linneaus, gingivitis, *hexetidine*

Abstrak

Gingivitis adalah salah satu jenis penyakit periodontal dan bersifat kronis. Faktor etiologi utama gingivitis adalah plak bakteri. Gingivitis yang tidak dirawat dapat menyebar ke jaringan periodontal di bawahnya menjadi periodontitis dan mengakibatkan kerusakan jaringan. Salah satu perawatan gingivitis adalah pembuangan plak, dibantu dengan obat kumur antibakteri untuk mengurangi plak bakteri. Daun *Lawsonia inermis* L. telah digunakan oleh penduduk pedesaan di Indonesia untuk penyembuhan luka di kulit dan dilaporkan menunjukkan efek antibakteri secara *in vitro* dan *in vivo*. Penelitian ini bertujuan untuk mengetahui pengaruh infusa daun *Lawsonia inermis* L. pada penyembuhan gingivitis. Sebanyak 63 pasien gingivitis dibagi menjadi 5 kelompok diinstruksikan untuk berkumur dengan infusa daun *Lawsonia inermis* L. dalam 3 konsentrasi (50000 µg/mL, 10000 µg/mL, 5000 µg/mL), larutan *hexetidine* 0,1%, dan akuades sebagai kontrol. Perdarahan gingiva saat probing diukur dengan *Papilla Bleeding Index* (PBI). Hasil penelitian menunjukkan bahwa penurunan PBI pada infusa daun *Lawsonia inermis* L. dengan konsentrasi 10000 µg / mL (80%) lebih kuat dari *hexetidine* 0,1% (76%). Sebagai kesimpulan, infusa daun *Lawsonia inermis* L. efektif menurunkan PBI lebih baik daripada *hexetidine*, daun *Lawsonia inermis* L. efektif untuk mengobati gingivitis.

Kata kunci: *Lawsonia inermis* linneaus, gingivitis, *hexetidine*

INTRODUCTION

Gingivitis is one type of periodontal disease especially found in the developing countries and it was chronic in nature. The main etiological factor of gingivitis is bacteria plaque wich can damage tissue

attachment or periodontium; early on gingiva. The inflammation on chronic gingivitis will spread to a deeper attachment apparatus, and it can become periodontitis. If attachment apparatus is damage, tooth will be unstable, and it is easy to lose.[1]

The inflammation of periodontium can occur from various kinds of causal factors as bacterias and traumatics. Accumulation of microorganism as plaque attached on tooth surface constitutes the main cause of inflammation. Severe other factors also as trigger in periodontal disease, such as calculus, poor oral hygiene, crowded tooth, caries, unproportional and poor tooth restoration.[2]

The major principle treatment of gingivitis eliminated the etiological factors, to reduce or remove inflammation, so that it gives a chance on gingival tissues to repair. Treatment for gingivitis healing entity to plaque removal; scalling; improving plaque control regiment; filling of caries; orthodontic treatment; treatment of poor and unproportional dental restoration.[3]

Material compound contains of an antibacterial agents wich is needed to help the removal of the inflammation to prevent bacterial growth and reduced bacterial concentration in dental plaque.[4] An antimicrobial agent application on gingivitis patient evidence to reduce pocket's depth, pathogen periodontal's bacteria, and maximal treatment.[3]

Plant of *Lawsonia inermis* Linnaeus (*L. inermis* L.), is generally referred as *inai* (Indonesia) or henna which has nature value properties of wound healing. The leaves of *Lawsonia inermis* L. were familiar used by certain Indonesian villagers for wound skin healing. Usage of this leaf usually by pulverized and put it direct on wound area of skin, then wraped with gauze or cloth. It was anticipated that leaves of *Lawsonia inermis* L. have an antibacterial agent in nature so that it can accelerate skin wound healing. Temporary assumption, if leaves of *Lawsonia inermis* L. can cure skin wound, therefore it can also be utilized for wound healing in oral cavity included gingivitis.[5]

In general, clinical feature of gingivitis marked by gingiva appears as reddish, swelling with soft consistency, trend to bleeding if it is touched, and it exists plaque and calculus.[1] It is important to remove causal factors that trigger gingival damage.[6]

Antimicrobial compound works by obstructing growth or killing microorganism by various mechanisms; in a general way, their target regions are cell wall, ribosom, membrane of cytoplasm, and region of nucleic acid replication. There are inhibitions on cell wall formation or interference biosynthesis of some essential proteins, disturbances on deoxyribonucleic acid (DNA) metabolite, and change of microorganism cell membrane normal function. Interference on formation cell wall included disturbances of mucopeptida synthesis. Positive Gram bacteria especially sensitive to the antibiotic which inhibited the formation of mucopeptida, since mucopeptida protects cell wall which consists of thick relative mucopeptida layer, to back up cytoplasma's struc-ture. The layers of mucopeptida are known in se-veral names such as murein, glycopeptide or pepti-doglycan.[7]

Lawsonia inermis Linnaeus leaves have a lot of names that variably-different. In Indonesia known as 'inai' or 'pacar kuku'.[8] In England is known as *henna* or *camphire*. In Sanskrit it is known as Medayantika [9] (Figure 1).

Figure 1. A. Plant *Lawsonia inermis* L.[10] B. *Lawsonia inermis* L. from Batam Island

Lawsonia inermis L. is a flowering plant, it is native to tropical and subtropical regions of Africa, Southern Asia, and Northern Australasia in semiarid zones. *Lawsonia inermis* L. is a tall shrub or small tree, 2-6 m high. It is glabrous, multibranched with spine tipped branchlets. Leaves are opposite, entire, glabrous, sub-sessile, elliptical, and broadly lanceolate (1.5-5.0 cm x 0.5-2 cm), acuminate, having been depressed veins on the dorsal surface.[9]

In research of broad-spectrum antibacterial activity from traditionally used Indian medicinal plants, 66 ethanolic plant extracts were screened against nine different bacterias. Of these, 39 extracts demonstrated activity against six or more test bacterias. Twelve extracts showed broad-spectrum activity were tested against specific multidrug-resistant (MDR) bacteria, methycillin-resistant Staphylococcus aureus (MRSA) and extended spectrum beta-lactamases (ESbetaL)-producing enteric bacteria. In vitro efficacy was expressed in terms of minimum inhibitory concentration (MIC) values of plant extracts. MIC values ranged from 0.32-7.5 mg/ml against MRSA and 0.31-6.25 mg/ml against ES betaL-producing enteric bacteria. The overall activity against all groups of bacteria were found in order of *Lawsonia inermis* L. In addition, these extracts showed synergistic interaction with tetracycline, chloramphenicol and cyprofloxacin against *S. aureus* and/or *Escherichia coli*.[11]

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The ethanolic extracts of more than 12 plants were found nontoxic to sheep erythrocytes and nonmutagenic, determined by Ames test using *Salmonella typhimurium* test strains (TA 97a, TA 100, TA 102 and TA 104). Ethyl acetate, acetone and methanol fractions of more than six plants indicated that the active phytocompounds were distributed mainly into acetone and ethyl acetate fractions, whereas they were least prevalent in methanol fractions as evidence from their antibacterial activity against MDR bacteria.[11]

Nayak et al.[12] have been studying the ethanol extract of *Lawsonia inermis* L., was used to evaluate the wound healing activity on rats using excision, incision and dead space wound models. The extract-treated animals showed 71% reduction in the wound area compared with control was 58%. Histological studies of the tissue obtained on day 10 from the extracttreated group showed the increasing well organized bands of collagen, more fibroblasts and few inflammatory cells where compared with control showed inflammatory cells, scanty collagen fibres and fibroblasts. Enhanced wound contraction, increased skin even though breaking strength on the other side, hydroxyproline and histological findings suggest the use of *Lawsonia inermis* L. in the management of wound healing.

In *Lawsonia inermis* L. leaves extract were found phytochemistry compound which have antibacterial activity properties in nature, as flavonoids, essential oils, saponins, steroids, triterpens, and tannins.[13] This rsearch aimed to know the effectiveness of *Lawsonia. inermis* L. leaves infusion to gingivitis healing, compared with 0.1% *hexetidine* solution.

MATERIALS AND METHODS

This research is a clinical trial to determine the effectiveness of *Lawsonia inermis* L. leaves infusion toward the decreasing in plaque index and gingivitis healing in humans. Research subjects were 63 patients with gingivitis aged 15 to 56 years, did not suffer from systemic diseases, and had chronic gingivitis among 2 until 4 teeth. Research was performed at Periodontic Department, Dental and Oral Education Hospital Faculty of Dentistry Trisakti and University of Indonesia, Jakarta.

Infusion is produced by heat waterbath until 90^{o} C. Dry simplisia of *Lawsonia inermis* L. leaves as many as 10 g were inserted into canister, added by water with the comparison 10: 100 (10 g simplisia in 100 mL waters). Canister was inserted into *waterbath* and let it up to 15 minutes on the temperature 90^{o}-98^{o} C while swirled; then canister was excluded from waterbath. Simplisia was squeezed and screened by flannelette. Infusion that produced in concentration 10% (100 mg/mL). Infusion then mixed up with 5% honey, and 0.008% menthol oil to give tastier aroma as gargle. Infusion divided into 3 groups of concentration, which were 5, 1, and 0.5% (equal with 50000 µg/mL, 10000 µg/mL, and 5000 µg/ mL).

Each concentration of infusion group was inserted into dark bottle 100 mL. As many as 63 subjects divided into 5 groups of concentration (50000 µg/mL, 10000 µg/mL, and 5000 µg/mL). Group 4, given by rinse contained hexetidine 0.1% as positive control. Group 5, given by aquabidest as negative control.

Patients who had given an explanation intention and effect of research, could sign a letter of informed consent as an agreement of the treatment. Papilla Bleeding Index was scored on tooth with gingivitis. Each subject was given 1 bottle contained 100 mL material solution tests.

Subjects were explained about manner in rinse with 10 mL material tests (1 full tablespoon). Rinse will be performed twice a day, in the morning and at night time after brushing teeth. Material tests were rinsed to all oral cavities, to right, left, onto and downwards on the region of vestibulum firmly, and was emphasized especially on the region of gingivitis that was analyzed. Rinse was done up about 30 seconds, then it was discarded. After 5 days, subject was instructed to return for controlling. Since treatment, subject was not permitted any other rinse material. On the 6th day score of PBI was registered. Patients who did not return for controlling after the 6th day, were not in following as subject of research. This clinical research used '*double mask*' method that examiner and subject did not know material tests contents.

To obtain score of PBI, periodontal probe was inserted into gingival sulcus until bottom without pressure. Probe was inserted on middle of marginal gingiva, then moved or swept coronally towards crest interdental papilla. Bleeding score after 20 until 30 seconds, while all gingival sulcus was probed. Scoring was obtained in perform at 4 levels which were 1, 2, 3, and 4. Scoring performed on oral and facial aspect, and selected from the highest score. Total score was noted as a 'number of bleeding'. Papilla Bleeding Index was scored by dividing total number of bleeding to number of total papills was checked.

RESULTS

Before treatment of 50000 µg/mL concentration, marginal and interdental gingiva on teeth 32, 31, 41, 42 were reddish and swelling; and bleeding on

probing on teeth 41, 42. After treatment, marginal and interdental gingiva on teeth 32 to 42, showed the reducing of reddish and swellling, and there was no bleeding on probing. Before treatment of 10000 µg/mL concentration, marginal and interdental gingiva on teeth 32 to 42 showed red color and swelling. After treatment, marginal and interdental gingiva on teeth 32 to 42, showed red color and swelling disappeared (Figure 2).

Figure 2. A. Before treatment, marginal gingiva and interdental papilla 32-42 appeared in red and swollen. B. After treatment by 10000 µg/mL concentration, marginal gingiva and interdental gingiva 32-42, red and swelling disappeared

Before treatment of 5000 µg/mL concentration, marginal and interdental gingiva of teeth 32 to 42 showed red in color and swelling. After treatment, marginal and interdental gingival of teeth 32 to 42, showed reducing red color and swelling, and consistency was rather firmly. Before treatment of hexetidine 0.1%, marginal and interdental gingiva teeth of 32 to 42 appeared red in color and swelling. After treatment, marginal and interdental gingival teeth of 32 to 42, showed red color and swelling disappeared (Figure 3).

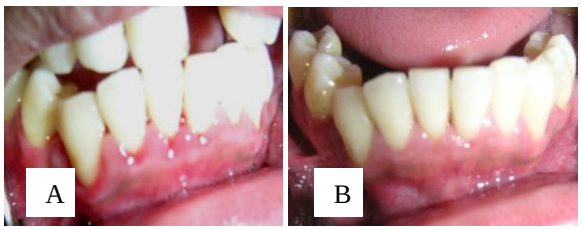

Figure 3. A. Before treatment, marginal gingiva and interdental papilla 32-42 appeared in red and swelling. B. After treatment by hexetidine 0.1 %, marginal gingiva and interdental gingiva 32-42, red and swelling were reduced

The decreasing of PBI percentage majority existed on sample of *Lawsonia inermis* L. leaves infusion at 10000 µg/mL concentration (80%), was stronger than hexetidine 0.1 % (76%). Thus the effectiveness of *Lawsonia inermis* L. leaves infusion at 10000 µg/mL concentration was better than hexetidine 0.1%.

DISCUSSION

Dental plaque is the main aethiological factors causes periodontal disease, begin from gingivitis, since plaque contains of various kinds of bacteria that can lead to periodontal destruction. Besides plaque as the main cause, gingivitis is also being triggered by factors that contributes to plaque accumulation, as calculus, poor oral hygiene, crowded tooth, caries, unproportional construction and poor dental restoration.[2]

Untreated gingivitis, the inflammation will spread to deeper supporting tissues which is periodontal ligament, cementum, and alveolar bone, as attachment apparatus. Destruction on supporting tissues will lead to loss of attachment and loss of tooth. Plaque was attached firmly on tooth, gingiva and also on oral appliance as fillings, prothesis, and orthodontic appliance. Gingivitis healing can be performed by removing plaque with more effective manner through mechanical method as with toothbrush, dental floss, oral irrigation, and another interdental cleaner tool. Enhancing with the removal of calculus, and other contributing factors support plaques accumulation.[4]

Besides with mechanical method, healing of gingivitis can be helped and fastened by antibacterial material purpose that avoids plaque growth and also reduces a number of bacteria in plaque. Antibacterial material is generally packed as liquid gargle. In periodontology, gargling is used to help the removal of plaque, reducing inflammation and helping for healing after periodontal surgery. Gargling can also be used on the treatment of wound, and help for healing another surgery in oral cavity.[15]

This research, used *Lawsonia inermis* L. leaves infusion in 3 concentrations (50000, 10000, and 5000 µg/mL) compared with 0.1% hexetidine. In this research, material research was made in infusion form because it is necessary to use fresh condition of gargle without chemical material as dissolving agent. Negative control group using aquabidest with reason that rinsing with aquabidest can also help in plaque removal. In addition, aquabidest only used for rinsing, and it is not harmful if swallowed.

Score of PBI was used as parameter of gingivitis healing. The result showed that there were signifycant differences between probing in early PBI, and final PBI. PBI Graphic profile showed PBI decreased in all groups. The largest decreasing existed on group concentration was 10000 µg/mL. Sharp decreasing on placebo group. PBI percentage decreased majority existed on group concentration 10000 µg/mL, then followed by concentration group

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50000 µg mL, stronger than 0.1% hexetidine. The least decreasing effect showed on group concentration 5000 µg/mL and on placebo group. The decreasing of PBI score showed that group concentration 10000 µg/mL could make gingivitis treatment stronger than 0.1% hexetidine (Figure 4).

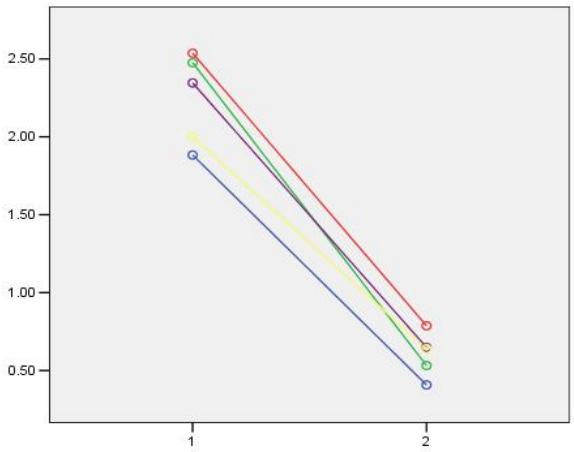

Figure 4. Papilla Bleeding Index Graph Profile.1. Purple = concentration of 50000 ug/mL, green= 10000 ug/mL, red= 5000 ug/mL, blue= hexetidine 0.1%, yellow= placebo; 2. The vertical line = PBI score; horizontal lines: 1= early score, 2 = final score; 3. PBI decreasing was found in all groups. Steepest decline in the group there was a concentration infusion 10000 ug/mL (green), was steeper than the 0.1% group hexetidine (purple)

Application concentration 10000 µg/mL resulted PBI decreased and better than concentration 50000 µg/mL. It was clear in the analysis on phytochemical *Lawsonia inermis* L., that the leaves contain of compound groups of phenol, alkaloids, glycosids, fla-vonoids, and tannins. *Lawsone* (*2-hydroxy 1,4-naphthoquinone*) is a main component as bioactivity agent.[11,16] Other compounds are also available in *Lawsonia inermis* L. leaves such as *p coumaric, 2-methoxy 3 methyl 1,4-naphthoquinone, apiin, lawsone, apigenin, luteolin, and cosmosiin.* [16-20]

Refering to bioactivity material content of leaf *Lawsonia inermis* L. therefore a large component part, it is phenolic group such as (*E*)*-methyl cinnamate, p-coumaric, 2-methoxy 3 methyl 1,4-naphthoquinone, apiin, lawsone, apigenin, luteolin, and cosmosiin* that has an iritant factor. As well as saponin compound group, it has broad spectrum activity as anti-bacterial agent, and it works as solvent that can irritate to mucous membrane. That material on high concentration or up to its therapy concentration causes iritant on epithellium especially gingival epithellium, avoid healing process. Meanwhile insufficiently dose did not have healing effect. Thus infusion concentration 10000 µg/mL showed the best of all optimals to reduce PBI.

To determine the success to control inflammation and reduce disease development, bleeding was a very good indicator. Although bleeding on probing not such the most specific or sensitive health measure, but it has strong negative correlation to disease development. If bleeding is not found in all parts on oral cavities, it reflected of good control plaque and disease handle and it will avoid of periodontal disease progression. It is concluded that *Lawsonia inermis* L. leaves infusion are effective to treat gingivitis, thus, it is used as gargling to help gingivitis healing.

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