A Design Proposal

THE EFFECT OF EXTRACTED *Averrhoa bilimbi* MOUTHWASH AS AN ADJUNCTIVE THERAPY FOR GINGIVITIS:

A Randomized Control Study in Indonesia

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AFFIRMATION

I certify that this report is based on my own work and that I have acknowledged the work for others appropriately in accordance with the regulations pertaining to academic work at the University of Melbourne. I declare that this work had not been previously submitted as assessment at this or any other institution.

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ABBREVIATIONS/ACRONYMS

ADA	: American Dental Association
BFRSS	: Behavioural Risk Factor Surveillance System
CHD	: Coronary Heart Disease
CPITN	: Community Periodontal Index of Treatment Needs
CRP	: C-Reactive Protein
NHANES	: National Health and Nutrition Examination Survey
NSAF	: National Survey of America's Families
PBI	: Papilla Bleeding Index
PMN	: Polymorphonuclear Leukocyte
PLIS	: Plain Language Information Statement
SES	: Socioeconomic Status
WHO	: World Health Organization

ABSTRACT

BACKGROUND

According to The World Oral Health Report (2003), oral disease has become a global public health problem (Petersen, 2003). In Indonesia, dental disease is the the sixth most common disease group in the community, with prevalence as high as 60 percent of (adult) population (Mangundjaja and Auerkari, 1999). The main aim of gingivitis treatment is to eliminate the etiologic factor, plaque, by brushing (Perry, 2006). However, inadequate brushing techniques result in imperfect maintenance and for this reason mouthwash has been recommended as a supplementary treatment (Eley et al, 2010).

Antimicrobial agent in mouthwash helps reduce bacterial build-ups on dental surface. Chlorhexidine, the gold standard of antimicrobial agents, can cause a variety of adverse effects such as extrinsic stain on teeth, olfactory disorders, and oral mucosa irritation because of its alcohol-based ingredients (Quirynen et al, 2005). Mouthwash with *Averrhoa bilimbi* extract may have a clinical benefit in reducing the severity of gingivitis and averting periodontitis which needs to be tested in a well designed epidemiological study.

AIM

This research project aimed is to design an epidemiological study protocol to investigate the efficacy of mouthwash containing extracted *Averrhoa bilimbi* as an adjunctive therapy in gingivitis.

METHODS

Prospective and non-prospective study designs were evaluated based on the relationship between exposure (use of *Averrhoa bilimbi* mouthwash) and outcome (severity of gingivitis) in the observation.

A prospective cohort study can be considered as a feasible option for design. The prospective study could incorporate the comparison of participants groups with different risk factors and would follow them over time to determine the reduction in the severity of gingivitis. Participants could include those that are thought to be have possible risk factors for gingivitis, while the other group could include participants that do not have associated risk factors. However, the aim of this study is to design a protocol to assess the efficacy of a mouthwash-

treatment for individuals with gingivitis, rather than a study to assess a preventative measure of gingivitis. Therefore, all subjects should have gingivitis at baseline. A retrospective cohort study and a case-control study are also less feasible since the mouthwash containing extract *Averrhoa bilimbi* is a new treatment and has not been previously used.

Cross-sectional design could potentially be considered since this design is cheaper and quicker compared to other designs (Elwood, 1998; Woodward, 2005). However, there will be two time periods for the collection of data (at baseline and day 30) which effectively renders this design not suitable for the study. Most of the studies which aim to test new drugs/medicines use a randomised controlled trial design. In this design, participants having gingivitis will be randomised into either an intervention or a control groups.

RESULT: FINAL PROTOCOL

The proposed study will be a randomised controlled trial with double blinding. The participants in this study will be selected from registered patients attending Periodontic Department at Trisakti University Dental Hospital, in Jakarta, Indonesia. Subjects will be randomly assigned by an independent statistician into intervention and control groups. The intervention group will receive active treatment (i.e. mouthwash containing extract *Averrhoa bilimbi*), while the control group will receive placebo (i.e. mouthwash containing saline solution). Both groups will receive an identical inpatient treatment of dental cleaning at randomization and 7 days post-randomisation. Each arm will have 49 participants, who will be followed for 30 days based on the average duration of gingivitis healing process. Data will be collected using Papilla Bleeding Index (PBI) which measure the bleeding severity, while asculin test and count agar plateswhich identify and count number of gingivitis plaque bacteria. Analysis will include desciptive and paired student t-test to examine the mean difference between intervention and control groups, before and after treatment. The outcome of this study will be analysed using Intention To Treat analysis (ITT).

CONCLUSION

Gingivitis remains a worldwide health problem which affects both developing and developed countries. The lack of recognition of early stage gingivitis symptoms,, a lack of education, and socio-economic factors are considered as factors that result in delays in the prevention of gingivitis. The use of *Averrhoa bilimbi* extract in reducing severity of gingivitis is expected to provide more benefits to community, since it is affordable and easy to access.

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1. INTRODUCTION

Gingivitis is an inflammatory lesion caused by an accumulation of plaques and is limited to the gingival tissue of the oral mucosa. Gingivitis can spread to periodontal tissues such as periodontal ligament, cementum, and alveolar bone (Fiorellini et al, 2006). Progression of gingivitis is a major underlying cause of loss of teeth in the majority of adult cases as it is associated with chronic oral inflammation and also increases the risk of some systemic diseases, such as cardiovascular disease and diabetes mellitus (Carranza and Takei, 2006). The influence of systemic diseases on periodontium are that systemic diseases effectively lead to a reduction in defense mechanisms, resulting in an increase in an individual's susceptibility to infections, which potential can trigger the development of periodontal disease (Gurenlian, 2006; Klokkevold & Mealey, 2006).

Paleontologic studies indicate that gingivitis existed in the early of 2000 B.C., today it remains a worldwide health problem (Carranza & Perry, 1986). The Third National Health and Nutrition Examination Survey (NHANES III, 1988-1994) in the United States found that around 54% adults were suffering from gingivitis on at least three or four teeth (Beck & Arbes, 2006). In Indonesia, according to the latest published estimates, gingivitis ranks sixth among the most common disease, with 60% prevalence in the (adult) population (Mangundjaja & Auerkari, 1999; Indonesia National Household Survey, 2004).

Standard treatment for gingivitis involves removal of plaque by proper brushing or by dental scaling and root planing for severe plaque and dental calculus (Pattison & Pattison, 2006). Unfortunately, the brushing method is limited in its capacity to eliminate dental plaque and effectively kill bacterial pathogens. The use of antiseptic mouthwash has been recommended as a strategy for effective teeth cleaning, particularly for cleaning of the interproximal area (space between teeth) and for cleaning the posterior aspect of teeth (Eley et al, 2010).

Some mouthwashes contain a main ingredient that is sourced from an extract of *Averrhoa bilimbi*, a native plant to Indonesia. It has been demonstrated that *Averrhoa bilimbi* a potent antibacterial agent against some Gram-positive and Gram-negative bacteria that colonise in the mouth (Pushpakumara; Zakaria, 2007). Despite knowledge of this antibacterial effect, there is limited published clinical evidence that can scientifically validate the effectiveness of *Averrhoa bilimbi* extract in reducing the severity of gingivitis.

The aim of this research project is to develop a study protocol to investigate the effectiveness of a mouthwash containing extracted *Averrhoa bilimbi* in (i) reducing the severity of gingivitis and (ii) reducing the number of gingivitis plaque bacteria. The study is to be conducted in Jakarta, Indonesia. A range of potential study designs will be considered in order to determine the most appropriate design. Considerations will include: participant eligibility; the intervention/therapies for the active and control groups; the outcome of interest; length of follow-up (for a prospective design); and the sample size required to detect clinically significant benefits of *Averrhoa bilimbi*. The project will also consider the available methods used to measure presence and severity of gingivitis including the Papilla Bleeding Index (PBI) as well as Eskulin test and Agar Plate Count to identify and calculate number of gingivitis bacteria.

The method of data analysis, include the use of descriptive analysis for measurement of associations between treatment and outcomes will also be proposed. This protocol development will allow for a formal scientific assessment of the impact of *Averrhoa bilimbi* in reducing the severity of gingivitis and the number of gingivitis plaque bacteria.

2. BACKGROUND

According to The World Oral Health Report in 2003, oral disease has become a global public health problem which corresponds with changing dietary patterns across all population groups (Petersen, 2003). Changing dieatry patterns include an increased consumption of unhealthy foods, such as those foods high in sugar and fat, and increased consumption of alcohol, as well as the use of tobacco products (Petersen, 2003). One of the most common oral diseases is that of gingivitis or inflammation on gingiva. Gingivitis is found in all age groups, and its symptoms are readily observable in children. Chronic gingivitis if remaining untreated may lead to more severe conditions such as heart disease and metabolic disorders (Duperon & Takei, 2006; WHO, 2011).

Multiple factors have been known to cause gingivitis, therefore prompt treatment and appropriate management is required to control the prevalence of the disease (Hinrichs, 2006). Lower socioeconomic status is associated with an increased risk of gingivitis, as are other risk factors such as ageing and poor dental hygiene. This increased risk in lower SES population groups can be attributed to lack of access to nutritious foods and high prevalence of smoking and excessive alcohol consumption (Plasschaert et al, 1978; Paulander et al, 2003). Nevertheless, a combination of poor knowledge of oral health and high out-of-pocket expenses for dental services inhibits early treatment that a majority of gingivitis cases. In these cases gingivitis rapidly progresses to periodontitis whereby the inflammation penetrates in to the supporting structures of the affected tooth resulting in the increased potential of dental bone loss (Taggart and Perry, 2007).

Adjunctive therapy to stop the inflammation is required prior to the main treatment of medically assisted plaque removal. Mouthwash can represents as an effective and easy-to-use adjunctive therapy in gingivitis treatment. Mouthwash with *Averrhoa bilimbi* extract may have a potential clinical value to reduce gingivitis severity and avert periodontitis. Thus it is important to investigate the health effects of *Averrhoa bilimbii*-based mouthwash in a well designed epidemiological study.

2.1 Gingivitis

Gingivitis is an inflammation on gingiva and can be recognized by gum mucosa (tissue) that bleeds easily; changes in color (increased redness); looks swollen and forms an increase amount of exudates, (Fiorellini et al, 2006). It is perceived to be the most common human disease due mainly to its asymptomatic and indistinctive nature in non-advanced stages. This presentation complicates effective prevention measures and early treatment efforts (Taggart & Perry, 2007). The main cause of gingivitis is plaque bacteria, that as an untreated condition can spread deeper into periodontal tissues resulting in damage to the attachment apparatus of tooth before eventually causing tooth loss (Armitage, 1994; Carranza & Takei, 2006)

The predisposing factors cause of gingivitis are dental calculus, dental stains and inadequate dental restorations (Hinrichs, 2006). Since plaque bacteria are firmly attached to tooth surface, they can only be removed by teeth cleaning using a toothbrush or an interdental cleaning tool (Perry, 2006). Treatment of gingivitis is intended to remove the etiological factor and aimed to initiate the natural healing process of gingiva (Perry et al, 2006). Untreated gingivitis can have implications for oral and systemic health.

A B C

Fig.2-1. Gingiva. A,Healthy Gingiva (gum). B, Gingivitis. C, Periodontitis (From Electro Medical System, 2011)

2.1.1.Oral Implications

In the early stage of gingivitis, tissue damage is only observable by a microscope, and disease progression is therefore largely asymptomatic to the patient (Fiorellini et al, 2006).

Before advancing to periodontitis, the damage is localized to the gingival tissues and presents in the form of easy bleeding, rednesess, gingival enlargement and halitosis in individuals with poor oral hygiene (Carranza et al, 2006).

In the subsequent stage, known as periodontal destruction, the inflammation spreads to the periodontal ligament and the alveolar bone, thus this stage is known as periodontal destruction or periodontitis, with periodontal pocket formation, alveolar bone resorption, and resulting tooth loss (Carranza & Takei, 2006). The long-term consequences of tooth loss can be serious as it disrupts aesthetic and mastication functions of teeth and has deleterious impact in physiological and general health.

2.1.2 Systemic Implications

Recent studies reveal unique association between gingivitis and systemic diseases. The association is a two-way road, whereby systemic host factors can reduce resistance to periodontal destruction and the presence of local bacteria can induce and worsen systemic diseases (Klokkevold & Mealey, 2006). Untreated gingivitis in the periodontal destruction stage can increase the risk of having a systemic disease, which alone has been shown to deteriorate oral health suggesting the key role of gingivitis in facilitating the vicious cycle between oral ill-health and systemic diseases.

A study by Tonetti et al (2007) shows that microorganism in periodontal tissues are associated with the development of systemic diseases, such as coronary heart disease and atherosceloris. Moreover, bacteria that are found in gingivitis can be a trigger for blood clots and plaque build-up that can lead to heart attack, stroke, and atherosclerosis (Gurenlian, 2006). In a prospective study by DeStefano et al. (1993) found that subjects in the periodontal destruction stage had a 25% increased risk of coronary heart disease (CHD), while younger subjects aged between 25-49 years had a 70% increased risk of CHD.

One well-described association between untreated gingivitis and systemic disease is plaque bacteria as a local host, the polymorphonuclear leukocyte (PMN) as the first line of defense mechanism, and C-Reactive Protein (CRP) as a predictor risk of CVD (Mealey & Klokkevold, 2006).

Untreated gingivitis in the periodontal destruction stage will result in bacterimias and induce the production of inflammation factors, such as TNF-α and Interleukin-6, which likely could stimulate the liver to create C-Reactive Protein (Genco et al, 1999). As a defence agent which will neutralize activities of plaque bacteria, PMN may potentially cause periodontal destruction. Moreover, periodontal disease could increase the risk of cardiovascular disease since oral infection increases the systemic inflammatory challenge to the vascular system, with potentially damaging consequences. For instance, patient in periodontal destruction stage have been shown to have high serum level of CRP, and levels of this particular inflammatory marker also correlate with measure of cardiovascular disease. An increase in PMN activity as a result of periodontal destruction stage may similarly have adverse effects on the cardiovascular system (Dowsett & Kowolik, 2001).

Diabetes mellitus is associated with periodontal disease. Diabetes mellitus patients with periodontitis will have a higher risk of poor glycemic control compared to diabetes mellitus patient without periodontitis (Dowsett & Kowolik, 2001). Studies by some researchers also show that untreated gingivitis in periodontal destruction stage can increase risk of premature birth and low birth weight (Offenbacher et al, 1996; Jeffcoat et al, 2001; Scannapieco et al, 2003).

2.2. Antimicrobial Agents

The main aim of gingivitis treatment is to eliminate the etiologic factor, specifically the plaque formed by colonizing bacteria (Perry, 2006). While simple treatment with regular brushing can remove plaque and prevent inflammation, inadequate brushing techniques and difficulties to reach some areas in mouth, such as proximal section and distal of last molar result in inadequate maintenance (Eley et al, 2010). In addition, antimicrobial agents are needed to ameliorate the inflammation by inhibiting the growth of bacteria and reducing the number of bacteria. The application of antimicrobial agents result in the reduction of pocket depth, reduction in the number of pathogenic bacteria, as well as will provide better treatment result (Perry et al, 2006).

The gold standard of antimicrobial agents in preventing the development of bacteria and plaque formation is chlorhexidine, which has been approved by American Dental Association (ADA) (Perry, 2006). Unfortunatelly, regular use of chlorhexidine can cause adverse effects, such as extrinsic stain on teeth, olfactory disorders, and oral mucosa irritation because of its alcohol-based ingredients (Quirynen et al, 2005).

These side effects have been found in other chemically derived antimicrobial agents, increasing the need for antimicrobial agents made from natural materials (Rateitschak et al, 1985). Tichy et al (1998), explored potential use of a variety of common plants in periodontal disease treatment and found that of the many beneficial antimicrobial agents in these naturally grown plants only a few have been clinically tested.

In Indonesia, where herbal traditional medicine has been widely used since many centuries ago. However evidence of effectiveness and safety is limited despite its wide accessibility and affordability of the traditional medicine compared to the conventional medico-technical medicine. The proposed study described in this protocol can be seen as an attempt to provide scientific evidence of such traditional treatments by investigating the clinical effect of mouthwash made from *Averrhoa bilimbi* extract as an adjunctive in gingivitis treatment.

2.2.1. *Averrhoa bilimbi*

Averrhoa bilimbi (cucumber tree) or in Indonesia known as *belimbing wuluh* is a tree which can easily be found in tropical areas (Orwa et al, 2009). *Averrhoa bilimbi* is a native plant from Indonesia generally used in cooking (Pushpakumara).

Studies have reported that *Averrhoa bilimbi* is traditionally used for treatment of cough, cold, itches, boils, rheumatism, syphilis, diabetes, whooping cough and hypertension in the region of Asia (Orwa et al, 2009). Additionally, other perceived benefits of *Averrhoa bilimbi* include the use of its leaves in treating skin irritation and swelling mumps, as well as for baby tonic. The essence of the fruit can be used as the alternative treatment for fever, inflammation, and hemorrhoid (Orwa et al, 2009). In many parts of Java, *Averrhoa bilimbi* fruits are mashed and placed on the bleeding gums to stop the bleeding and ameliorate inflammation, or boiled into liquid solution to use as a mouthwash (Santoso, 2011).

In an *in vitro* study by Zakaria et al (2007), the researchers found that dried leaves and fruits of *Averrhoa bilimbi* have an antibacterial effect against some Gram-positive bacteria, such as *S. aureus, S.epidermis, B.cereus, C.diphteriae, and* Gram-negative bacteria, *such as S.typhi, C.fuendii, A.hydrophila, P.vulgaris*. In addition, an antibacterial study by Huda et al (2009) found that the fruit itself has an antibacterial activity. The phytochemical screening of *Averrhoa bilimbi* fruit extract shows the existence of flavonoids, saponins, and triterpenoids as shown by methanol as a solvent (Huda et al, 2009).

Another study by Jantan et al (2010) found that *Averrhoa bilimbi* has antiinflammatory, antibacterial, astringent, antiscorbust, post partum protective, anti lipid peroxidative and anti atherogenic effects. As it has many advantages, *Averrhoa bilimbi* can be expected to be an effective agent to reduce gingivitis. Moreover, ease in obtaining *Averrhoa bilimbi* allows this material to be cheaper compared to other materials. Thus, all communities can afford to use the result of this study to cure gingivitis.

2.3. Rationale

The rationale for the study includes:

(1). The high prevalence of gingivitis in Indonesia which affects individuals quality of life.

(2). The knowledge that untreated gingivitis has the potential to affect both oral and systemic health resulting in potential cost burdens to patient's

(3). Indonesia has a wealth of biological and traditional plants that may contain potential materials for phytomedicines.

(4). *Averrhoa bilimbi* is a native plant and available in abundance

(5). *Averrhoa bilimbi* has an antibacterial activity, antiinflammation and astringent effects that may be potentially beneficial in gingivitis therapy

(6). Filling the gap in knowledge regarding the potential efficacy of *Averrhoa bilimbi* in gingivitis treatment.

2.4. Proposed Research project

2.4.1. Aims of the Research Project

The aim of this research project is to design a protocol to conduct an epidemiological study to investigate the effectiveness of extracted *Averrhoa bilimbi* mouthwash as an adjunctive therapy in the treatment of gingivitis.

2.4.2. Hypotheseis

a. Extracted of *Averrhoa bilimbi* mouthwash has an efficacy as an adjunctive therapy in gingivitis treatment compared to non extracted *Averrhoa bilimbi* mouthwash treatment.

b. Extracted of *Averrhoa bilimbi* mouthwash has an efficacy in reducing the severity of gingivitis

c. Extracted of *Averrhoa bilimbi* mouthwash has an efficacy in reducing the number of gingivitis plaque bacteria.

2.4.3. Objectives

a. Primary Objective

The primary objective of this research project is to design a protocol to assess the efficacy of mouthwash made from extracted *Averrhoa bilimbi* as an adjunctive therapy for gingivitis, compared to the standard treatment of mouthwash that contains no *Averrhoa bilimbi*

b. Secondary Objectives

The secondary objectives are :

1. To assess the efficacy of extracted *Averrhoa bilimbi* mouthwash in reducing the severity of gingivitis

2. To assess the efficacy of extracted *Averrhoa bilimbi* mouthwash in reducing the number of gingivitis plaque bacteria

3. METHODOLOGY

3.1. Consideration of Study Design

This section will critically analyse potential study designs with the aim of selecting a feasible study design that is suitable and feasible for the purpose of investigating the efficacy of mouthwash containing extracted *Averrhoa bilimbi* as an adjunctive therapy for gingivitis.

The following paragraphs outline the main features of the potential study designs.

a. Cohort Study

➔ Prospective

Cohort study design follows study participants with varying levels of exposure to an outcome over a specified period of time. Cohort studies have the objective of observing how outcomes differ by exposure group at the end of the study period. As such, this design is useful in describing gingivitis incidence, exploring risk factors and the etiology of gingivitis by following participants over time (Slade).

This design can be considered to test the efficacy of extracted *Averrhoa bilimbi* mouthwash in reducing the severity of gingivitis by comparing groups of participants with different risk factors and following them over time to determine the severity reduction of gingivitis. Cohort study typically requires the investigator to select two disease-free groups (exposed and unexposed), which means participants should be free of the outcome when they enter the study at baseline (Woodward, 2005; Chattopadhyay, 2010). Therefore, the selection of subjects consists of people who were thought to have possible risk factors for gingivitis, while the other group does not have possible risk factor for gingivitis (Woodward, 2005). However, the aim of this study is to treat individuals with gingivitis, not study a preventative measure of gingivitis. Therefore, all subjects should have gingivitis at baseline.

Moreover, the investigator cannot control the intervention, which means no control for procedure, doses, or how to use the active/in-active treatment. For instance, participants may use active/in-active treatment as much as they want which result in unproportional treatment between both groups. This unproportional treatment will cause bias and affect internal validity.

➔ Retrospective

Cohort study can be done retrospectively where group of people which may thought at risk can be defined and measured from past exposure. However, in this study, the mouthwash containing extract *Averrhoa bilimbi* is a new treatment and has not been previously used. Therefore, it is not possible to conduct retrospective study based on efficacy of this treatment compared to another.

b. Case-control Study

This design is feasible in exploring the factors that influence gingivitis and in assessing the etiology factor (Slade). In this design, particants will be selected based on whether they have a particular disease or not (gingivitis versus healthy gingiva) and the exposure from both cases and control groups will be compared.

However, case-control study is viewed as a retrospective design, which is not in accordance with the aim of the study to test the efficacy of a new drug. Therefore, all participants should have gingivitis at baseline, since these study groups are established based on the severity of gingivitis. Similar to the cohort study, in this design, the investigator can only observe the study without the intervention which may bias the result and affect internal validity. Therefore, this study is a less feasible study to be conducted.

c. Cross –sectional Study

Cross-sectional study, or survey design, has an aim to measure disease prevalence at a particular point in time. This design is widely used in surveillance of risk factors.

For instance, large surveillance systems such as National Health and Nutrition Examination Survey (NHANES), Behavioural Risk Factor Surveillance System (BRFSS), and National Survey of America's Families (NSAF) utilize this study design (Chattopadhyay, 2010). This study is feasible in identifying the risk factors for gingivitis and for measuring the prevalence of gingivitis.

This design would potentially also be cheaper and quicker compared to other designs (Elwood, 1998; Woodward, 2005). A cross-sectional design will only allow data collection at one point time through the use of questionnaires, survey's, or clinical assessment. This design compares participants who have gingivitis and rinse using extracted *Averrhoa bilimbi* with those who use placebo. The design also measures the exposure (extracted *Averrhoa bilimbi* mouthwash) against outcome (severity reduction of gingivitis) at a single point in time. In this study however, there will be two times of data collection (at baseline and day 30) which effectively renders this design not suitable for the study. Another issue in this design is the possibility of bias since the investigator has no control. In consideration of these limitations I would not consider this design as one that is suitable for the study.

d. Randomised Controlled Trial (RCT)

This design has been placed on top of the hierarchy of evidence and validity for the study, since the randomization of comparable participants into different treatment groups minimises confounding (Elwood, 1998). Most of the studies which aim to test new drugs/medicines use this design. In this design, participants having gingivitis will be randomised into either an intervention or a control group. The intervention group will receive active treatment, which is mouthwash containing extract *Averrhoa bilimbi*, while the control group will receive non-active treatment, which will be the saline solution. A limitation to this design is that participants may be able to differentiate between active treatment and placebo due to taste, which can effectively minimise the advantage of a double blind randomised controlled trial.

Study Design	Advantages	Disadvantages
Prospective Cohort	Feasible to explore risk factors and etiology of gingivitis	-Cannot control intervention - can prevent gingivitis, but cannot treat it
Retrospective Cohort		-Cannot control intervention -Cannot assess new treatment -Possibility recall bias
Case-Control	Feasible to explore association between gingivitis and active treatment	-Cannot control intervention -Cannot assess new treatment -Possibility recall bias
Cross Sectional	Feasible to measure gingivitis prevalence	-Measuring outcome and exposure in one occasion
Randomised Controlled Trial	Feasible to test the efficacy new treatment	-Can differentiate between active and non-active treatment

Table. 3-1. Consideration of Study Design

3.2. Consideration of Subjects Selection

3.2.1. Study Site

The selection of subjects and study site should be based on a geographic area of high prevalence of gingivitis to ensure the validity of the study.

3.2.2. Eligibility Criteria

Eligibility criteria determines whether a person could be allowed to participate in the study. Eligibility criteria are an important feature of the study design, as it establishes the validity of the study. Determinates for eligibility include considerations such as characteristics of participants, an individuals clinical or medical history and presence of disease such as gingivitis (Weng et al, 2010).

The selection of age in this study should reflect the target population of the project. Appropriate selection of age groups will minimise the influence of other factors which can potentially confound results. For instance, there are several types of gingivitis which is associated with sex hormones and puberty (Reddy, 2008). In contrast as age increases (generally more than 50 years), the risk of periodontal destruction stage also increases (Carranze et al, 2006).

Since this study aims to investigate the efficacy of a new treatment in reducing the severity of gingivitis, participants in both groups must have gingivitis. If gingivitis advances into periodontal destruction stage during the study, participants will be excluded since the procedures of therapy will be different and more complicated and may possibly result in surgery.

In order to increase the validity of the project, the risk factors for the periodontal destruction stage, such as smoking and systemic disease should be addressed. Studies in Indonesia, Japan, and Vietnam, demonstrate that smoking has a strong association with periodontal disease. In a study by Kasim (2001), it was found that long-term smoking will significantly increase the risk of having periodontal diseases. A cross-sectional study in Vietnam also reported that heavy smokers have almost eight times higher risk to have periodontitis than non-smoker (Do, 2001).

Similar to the two previous studies, a study conducted by Morita et al (2011) found that current and ex-smokers were more likely to have periodontal disease compared to non-smokers. Moreover, recent studies have shown that smoking can slow down the healing process which can impact the result (Perio.org, 2011). .

It is believed that periodontal disease also has a causal relationship with systemic diseases. Bacteria found in gingivitis can trigger blood clots and build-up plaque which can lead to heart attack, stroke, or atherosclerosis (Gurenlian, 2006). Moreover, some systemic diseases such as diabetes mellitus and cardiovascular disease can increase the risk of periodontal disease (Dowsett & Kowolik, 2001).

The use of orthodontic treatment in dentistry to prevent or correct dental malocclusion is helpful in restoring function and aesthetics of affected teeth. In fact, proper use and maintain appliances of orthodontic can reduce the risk of gingivitis (Kokich, 2006). For example, orthodontic treatment can correct dental malocclusion and overcrowding of teeth, thus resulting in a reduction of food debris and plaque formation and in effect preventing the development of gingivitis. On the other hand, it is perceived by some researchers that orthodontic treatment will increase the risk of gingivitis, especially in individual that have poor oral hygiene. An observational study conducted in Pakistan by Nasir et al (2011) concluded that there is a strong association between orthodontic treatment and progress of periodontal disease which was showed by increasing of CPITN index pre and after orthodontic treatment. Improper of orthodontic treatment and poor oral hygiene may in fact result in accumulation of plaques. Moreover, the appliances and bonds itself could be an ideal place for plaque retention increasing the risk of gingivitis (Reddy, 2008).

Antibiotics may be used in reducing or eliminating bacteria which cannot be removed only by brushing, scaling, or root planing. In dentistry, the administration of antibiotics can be systemic or localised (Jolkovsky & Ciancio, 2006). However, use of some antibiotics such as metronidazole, tetracyclines, and penicilins during the study period could bias the results of the study, since this aim of this study is to investigates the efficacy of extract *Averrhoa bilimbi* mouthwash as an antibacteria agent.

3.3. Consideration of Study Period

The participants will be followed-up to allow the investigator to measure the outcome. The duration of follow-up will be based on the length of time that the gingivitis healing process can be clinically detected.

3.4. Consideration of Study Procedures

Eligible participants will be examined at baseline. Once participants satisfy the eligibility criteria, they will receive information about the study objectives and procedures from the clinic staff and they will also be provided with printed materials relating to the study. Each participant will be required to sign a written consent form to ensure participation into the study is both voluntary and informed. In order to fulfill the aim of this study, participants will be clinically examined at baseline and on the final day to enable the comparison of the clinical effects of treatment with *Averrhoa bilimbi* mouthwash. The investigator will use either a dentist or dental hygienist to assess the clinical appearance of gingivitis

The problem of comparing active treatment and non-active treatment is to ensure that participants and investigator will not aware which group the participants have been assigned to. Participants or investigator awareness of the groups the participant have been assigned to would potentially lead to result bias.

3.4.1 Data Collection

3.4.1.1. Extracted *Averrhoa bilimbi*

Extraction and fractination of *Averrhoa bilimbi* will be conducted in accreditation Sciences Institute to ensure the purity of the extraction.

3.4.1.2. Clinical Assessment for Gingivitis Severity

Although the clinical symptoms of gingivitis are easily detected, it is not obvious how much gingival inflammation can be considered as gingivitis. According to Reddy (2008), there is no universal acceptance of indices in measuring severity of gingivitis. In previous studies of gingivitis, a variety of measurement tools have been used. Most of these indices use an ordinal scale to represent the severity of gingivitis. Ideally, a gingival index should be simple, quick to use, accurate, reproducible, and quantitative (Beck & Arbes, 2006). All gingival indices measure one or more of the following criteria such as gingival color, gingival contour, gingival bleeding, and gingival crevicular fluid (Beck & Arbes, 2006).

3.4.1.3. Clinical Assessment for Gingivitis Plaque Bacteria Concentration

The feasibility and validity of a specific tool should be considered in selecting an appropriate measurement tool. For sampling, plaque and saliva will be swabbed. Identifying and counting the concentration of gingivitis plaque bacteria in the oral mucosa can be done by examining the number of bacteria colonised on an agar plate using asculin test, (Salehi & Danaie, 2006).

3.5. Consideration of Sample Size

The sample size is calculated with the aim to minimise the type II error (Chan, 2003). It is determined by a calculation based on an estimation of mean difference between control and intervention group. The estimation of sample size calculation also be considered based on past clinical experience and literature review.

4. RESULT: FINAL PROTOCOL

4.1. Trial Design

A double blind, randomised controlled trial will be considered for conduction in the trial design as other observational studies cannot provide the high degree of validity as does a randomised controlled trial (Aschengrau & Seage, 2003). The implementation of randomisation of participants whether in the intervention or control groups would control bias more effectively compared to alternate study designs. Moreover, randomised controlled trial is the gold standard to test the efficacy of new treatment which is in accordance with the aim of this study . This trial will investigate the efficacy of mouthwash containing extracted *Averrhoa bilimbi* in severity reduction of gingivitis by comparing gingivitis participants who use or do not use extracted *Averrhoa bilimbi* mouthwash.

4.2. Selection of Subjects

4.2.1. Study Site

This study will be conducted in Trisakti University Dental Hospital, one of three dental hospitals in Jakarta. The prevalence of gingivitis in Jakarta is more than 60% (Indonesia National Household Survey, 2004). Trisakti University Dental Hospital has 12 dental units in Periodontic Department, with a daily average of 35 patients visits. Trisakti University Dental hospital has on-site panoramic and periapical x-rays and surgery rooms.

4.2.2. Inclusion Criteria

a. Between 18-48 years old

The participant age group selected will be between 18-48 years as gingivitis most commonly occurs in that particular age range. Below that range, the severity of gingivitis is influenced by puberty (for teens), and also because it will less feasible to conduct mouthwash clinical trial in younger childrens. Above the age of 48 was excluded, as the risk of having periodontitis or periodontal destruction stage is determined as an increased natural risk from the ages of 48 year and above.

b. Having gingivitis and at least having remaining six Ramfjord teeth

All participants must have gingivitis to be included in this study. Participants also must have at least six remaining "Ramfjord teeth" which consist of right maxillary first molar, left maxillary medial incisor, left maxillary first premolar (bicuspid), left mandibular first molar, right mandibular medial incisor, and right mandibular first premolar (bicuspid). The Ramfjord teeth commonly used in oral health research since it can represent the entire mouth (Chattopadhayay, 2011).

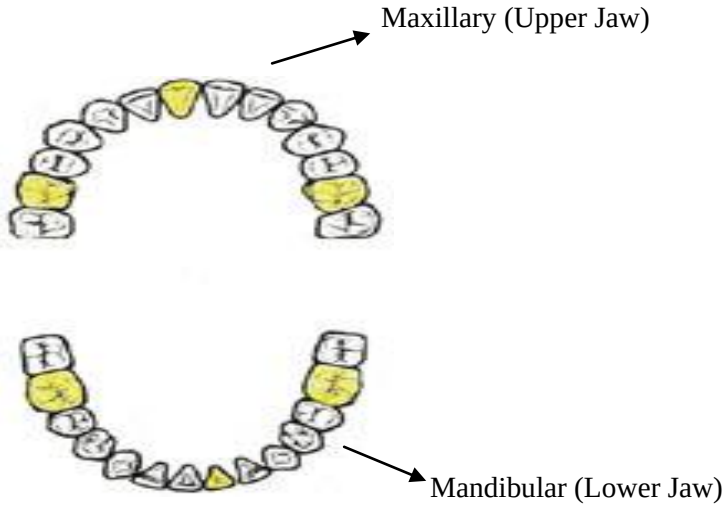


Fig. 4-1. Ramfjord teeth (from Rateitschack et al, 1985)

c. Having phone number

In order to ensure the compliance, participants must have phone number (landline or mobile phone) to be contacted daily.

4.2.3. Exclusion Criteria

a. Having periodontitis

This study aims only to assess the efficacy of new treatment in reducing severity of gingivitis. Therefore, periodontal destruction stage will be excluded.

b. Smokers

Current smokers and former smokers will be excluded in this study since findings from previous study showed the association between smoking and periodontal disease which is responsible in periodontal destruction and interference in the treatment healing process (Do, 2001; Dowset & Kowolik, 2001; Morita et al, 2011).

c. Women in pregnancy

Pregancy gingivitis or gravidarum gingivitis is a common type of gingivitis which occurs during the pregnancy due to changes in estrogen and progesterone hormone levels (Klokkevold & Mealey, 2006; Kapoor et al, 2010; Lavaurie, 2011). Pregnant women will be excluded in this study since during pregnancy, the risk of have gingivitis will always exist especially in case of the participant having poor oral hygiene (Carranza & Perry, 1986; Dowsett & Kowolik, 2001; Klokkevold & Mealey, 2006; Lavaurie, 2011). In addition, it is perceived that the condition of pregnancy will potentially bias the outcome measure.

d. Having a systemic disease

Participants who have systemic disease will be excluded from this study to ensure the homogenuity and validity of subjects, since systemic disease can induce periodontal destruction and disrupt normal wound healing process (Dowset & Kowolik, 2001).

e. Using orthodontic/denture appliances

Participants using orthodontic/denture aplliances will be excluded since the appliances can be an ideal place for plaque retention thereby increasing the risk of gingivitis. Moreover, orthodontic/denture appliances will complicate the measurement process when measuring severity of gingivitis since a periodontal probe will be inserted in gingival sulcus, while orthodontic/denture appliances will limit the movement of periodontal probe therefore biasing outcome measurements.

f. Taking any antibiotics during the study

In order to ensure the outcome as a result of the efficacy of active treatment, any antibiotics are prohibited during the study.

4.3. Study Period

According to Perry et al (2006), the evaluation of periodontal case can be assessed at about four weeks after completing scaling and root planing procedures. The reason for four weeks is to allow the healing process of both epithelial and connective tissues. Moreover, it will allow sufficient time for the antimicrobial agent to kill remaining pathogenic bacteria. Therefore, participants will be followed-up for 30 days.

4.4. Study Procedures

On the first visit, clinic patients will be examined to determine whether they satisfy the inclusion and exclusion criteria. This will be done by conducting a clinical assessment, such as anamnesis (memory or recall at the time of dental interview), oral screening (clinical appearance of gingiva), and measurement of gingival index. If a patient fulfills the inclusion criteria and if they are willing to attend the required procedures, they will be given information about the study and asked to sign an informed consent form to participate in the study. Reimbursement for travel costs during this study will be provided. The aim of this study will be explained to participants, and they will be randomly assigned to either an intervention group (standard treatments and active treatment mouthwash) or control group (standard treatments and non-active treatment mouthwash). Participants will be monitored daily during the study using contacts to ensure compliance of treatment.

On the first visit post-randomization, the severity of gingivitis of both groups will be examined using Papilla Bleeding Index (PBI). Both groups will receive the same standard treatments, which are scaling and root planing, and will be instructed to maintain appropriate oral hygiene at home. Both groups will be instructed to rinse approximatelly 15 ml of mouthwash for 30 second after brushing twice a day (after breakfast and before going to sleep at night). Both groups also will be given same toothbrush and fluoride toothpaste to reduce chance of bias and ensure the homogenuity of the standard treatment. The investigator will explain best practice in brushing technique and how to use the mouthwash.

Rinsing will commence on the same day on first visit post-randomization, and will be repeated every day until day 30. Participants will be recommended to avoid any food or drink one hour after using the mouthwash.

Bacterial sampling will be carried out two times. The first sampling will be done before participants receive standard treatment at baseline. While the second sampling will be done at day 30. Collecting bacteria from participants can be done by swabbing plaque and saliva. Subgingival plaque will be collected using currete and deposited in sterile tube containing distillated water (Amel et al, 2010).

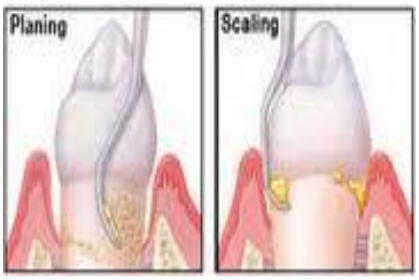


Fig. 4-2. Scaling and Root Planing (from Amel et al, 2010)

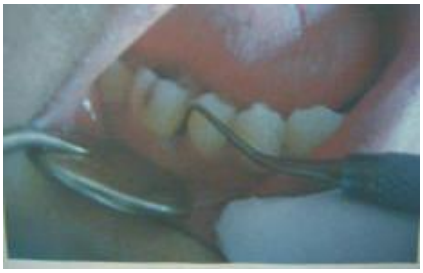


Fig. 4-3. Collecting subgingival plaque using currete (from Amel et al, 2010)

On the second visit or seven days post-randomization, participants will be required to visit Trisakti University Dental Hospital. This will ensure the investigator will be able to examine adverse events that may have occured. Participants will also be asked about the potential side effects of using the mouthwash and the result will be recorded. The clinical appearance of gingiva also will be examined. Scaling and root planing will be conducted again to eliminate irritant factors, while best practice home oral hygiene treatment techniques will also be reinforced.

At day 30 post-randomization (final assessment), the same procedures will be conducted, including examination of the clinical appearance of gingiva.

Measuring PBI to assess the severity of gingivitis and collecting plaque bacteria will be conducted to be compared before treatment (at first visit post-randomization).

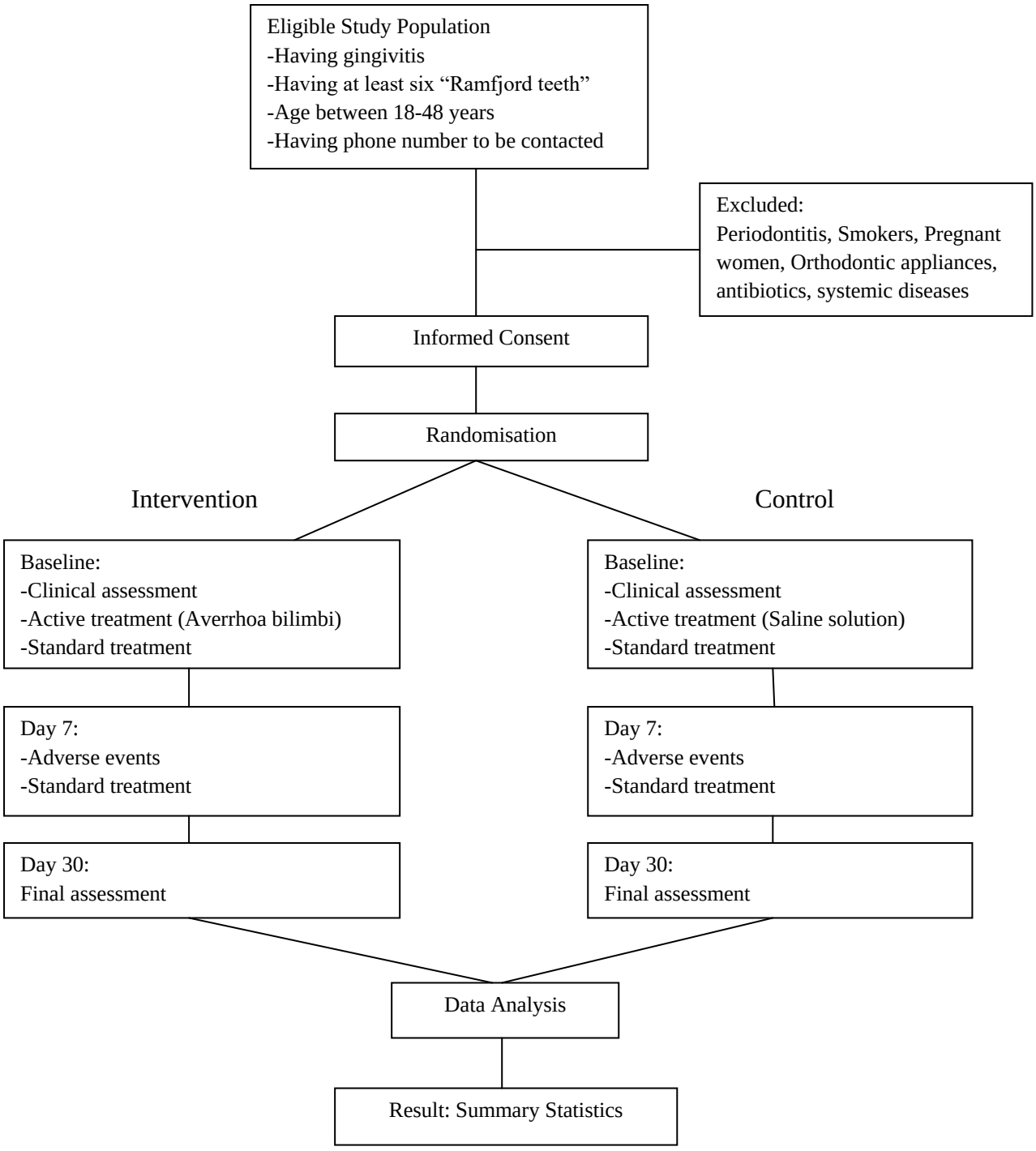
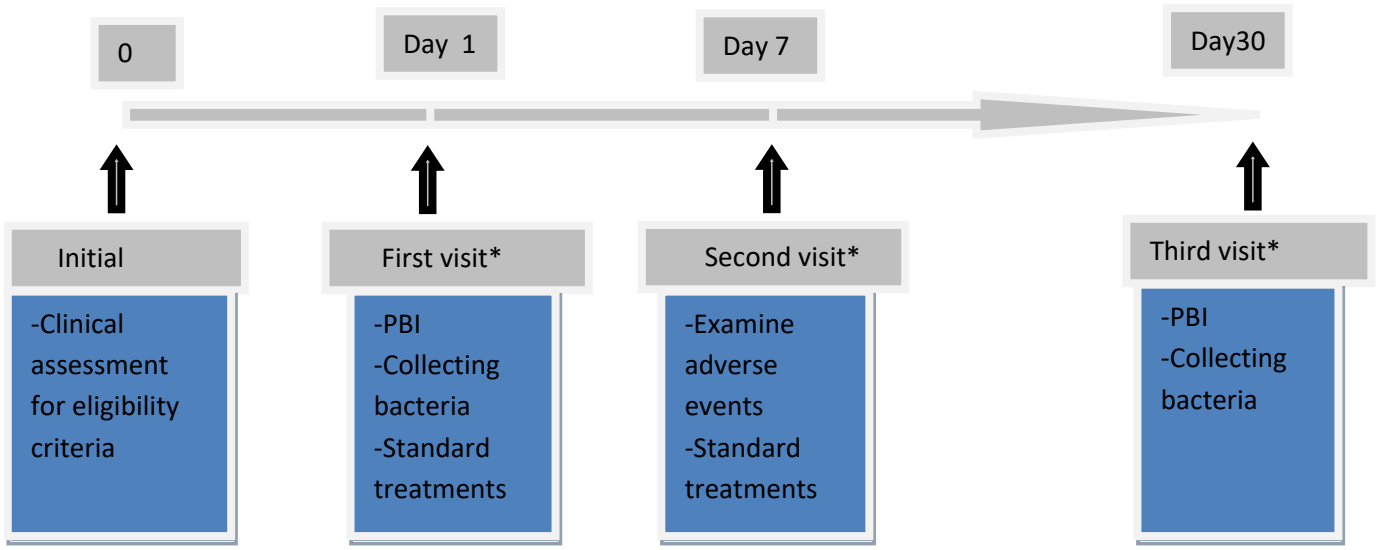


Fig. 4-4. Study Outline



*) – Post-randomisation

Fig. 4-5. Study Procedures

4.4.1. Logistic

Dental materials and instruments will be needed in this study, such as standard instruments (dental mirror, periodontal probe, dental explorer, and pinset), scaling instruments (supragingival and subgingival scalers, ultrasonic scalers), root planing instruments (gracey curetes and universal curetes), and cleansing and polishing instruments (rubber cups, bristle brushes).

Fig. 4- 6. Standard instruments (from Carranza et al, 2006)

Materials needed for collecting bacteria are sterille tube contains destillated water, petri plates, plate count agar, pipets, bunsen burner, and microscope.

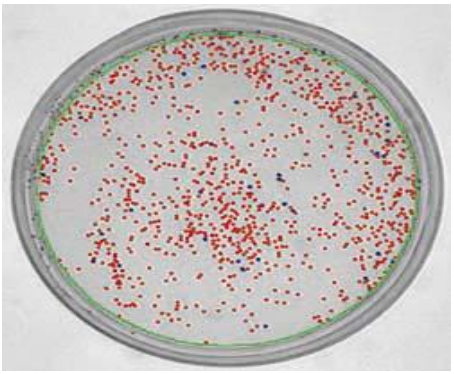


Fig. 4-7. Agar Plates (From Cayaoutte et al)

Fig. 4-8. Microscope (From Aunet)

4.4.2. Randomisation and Allocation Concealment

Randomisation and allocation concealment will be conducted in this study to ensure the validity of the study. Randomisation will be generated by using computer-derived system and will be supervised by statistician who has no contact with participants. Both, participants and investigator (dentist) will be blinded, which means they are unaware about the the treatment they receive or dispense. Both groups will receive identical bottles of mouthwash (extracted *Averrhoa bilimbi* versus placebo) which will be labeled and coded for identification purposes.

In order to support allocation concealment, the randomisation process will not be known by participants, treatment allocation will be sequentially-numbered and details of allocation will be placed in sealed opaque envelopes.

4.5. Data Collection

4.5.1. Extracted *Averrhoa bilimbi*

Extraction and fractination of Averrhoa bilimbi will be conducted in The Indonesian Institute of Sciences (Lembaga Ilmu Pengetahuan Indonesia). Based on findings from DIKTI, the most effective concentrate of *Averrhoa bilimbi* in inducing *Streptococcus mutans* is 16% (Dikti, 2009).

4.5.2. Severity of Gingivitis

Even though there are several indices that can be used in measuring the severity of gingivitis, this study will use Papilla Bleeding Index as a measurement tools (Rateitschak et al, 1985). This tool is choosen as gingivitis severity can be classified by the most obvious symptom, this being gingival bleeding. Other gingivitis symptoms can possibly lead to misclassification. The clinical assessment of gingival colour and texture is subjective in nature. For instance, if we use gingival colour or contour to measure the severity of gingivitis, it could lead to a bias since there are different colours or contours of gingiva in people from different races ethnic groups. Alternatively, gingival could be different because of variations in physiology, pathology or pigmentation. The clinical assessment of gingival bleeding is the most objective diagnostic tool for assessing the severity of gingivitis (Beck & Arbes, 2006).

This index is based on bleeding appearance following gentle probing on interdental papilla area. A blunt periodontal probe will be used to assess the bleeding index by inserting carefully periodontal probe into gingival sulcus at the base of interdental papilla on mesial aspects and moving coronally to the tip of papilla, and repeating on distal aspects of same papilla (Rateitschak et al, 1985; Reddy, 2008). The scoring criteria are (Rateitschak et al, 1985; Reddy, 2008) :

0 = No bleeding

1 = a single bleeding point appearance

2 = Single line of blood or several bleeding point appearance

3 = The interdental triangle fills with blood shortly after probing

4 = Profuse bleeding occurs after probing, blood flows shortly cover marginal sulcus

The score is obtained by totaling the score per affected tooth and dividing it by the number of examined teeth.

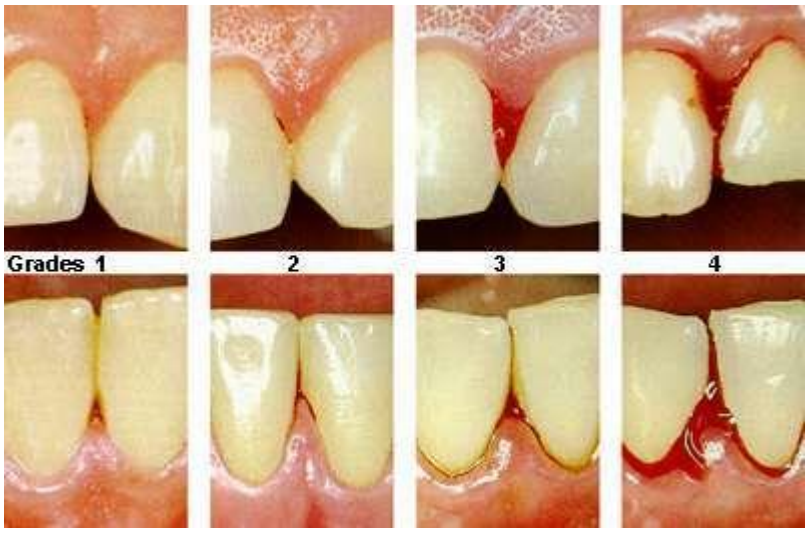


Fig. 4-9. Papilla Bleeding Index (from Rateitschak et al, 1985)

4.5.3. Gingivitis Plaque Bacteria Concentration

In order to count gingivitis bacteria concentration, agar plates count and asculin test will be choosen (Salehi & Danaie, 2006). The selection of agar plates count is because this tool simple and quick. While asculin test is a selective test used to isolate and identify members of group D *Streptococci* (including gingivitis bacteria, such as *Streptococcus mutans* and *S. sangunis*). The microbiological test will be done by using thioglycolate media at 37• Celcius for 24 hours.

After collecting sample, subgingival plaque will be dilluted with distilled water until the bacteria are dilute enough to be counting using microscope. Series of dilution should between 30 and 300 colonies (Reynolds & Farinha, 2005). The score is obtained by totaling colony count on agar plate and dividing it by total dilution of tube, multiply with volume plated (Eddleman, 1999)

4.6. Outcome

a. Primary Outcome

This will be the severity reduction of gingivitis which based on Papilla Bleeding Index (PBI).

b. Secondary Outcome

This outcome will be the reduction of gingivitis plaque bacteria concentration based on agar plate count and asculin test

.

4.7. Sample Size

This study with 49 participants for each treatment groups has an 80% power to detect the statistically significant treatment effect at 5% significance level, if the mean difference of severity reduction of gingivitis between control and intervention groups are 1.0 and 1.5 unit respectively. The difference of 0.5 unit in the estimated mean of severity reduction of gingivitis will constitute the smallest clinically relevant treatment effect between control and intervention groups.

Twenty percent extra participants will be allocated to each treatment arm to allow for potential loss to follow-up and treatment non-compliance, adjusting the total participants in this study to 98 subjects.

Sample Size Calculation Explanation

Sample size calculations have been conducted using STATA Software Version 11 as follows:

- The severity of gingivitis is measured in scale, from 0 to 4, which 0 as healthy gingiva, and 4 as the most severe condition of gingivitis.
- Mostly patients attend Periodontic Department with severity gingivitis score about 2.5 (moderate gingivitis)
- The estimation severity gingivitis score at first visit is 2.5
- The estimation benefit of Averrhoa bilimbi extract as an active treatment can reduce gingivitis severity from 2.5 to 1.0 (reduce 1.5 points). This estimation is based on previous study about chlorhexedine benefit as a gold standard treatment can reduce gingivitis after 30 days use from 50-60% (Perry, 2006).
- The estimation benefit in control group (in respect to standard treatment), can reduce gingivitis severity from 2.5 to 1.5 (reduce 1 points). This estimation is based on previous study about the effectiveness of standard treatment in reducing gingivitis severity as much as 40% (Perry, 2006).
- The estimation of standard deviation is based on normal distribution of population, which is 0.8
- Significance level is 95%
- Power of study is 80%
- Allowance for withdrawal is 20%
- Size of intervention group is 49
- Size of control group is 49
- Total : 98

4.8. Statistical Analysis

The data will be used to analyse the effect of extracted *Averrhoa bilimbi* mouthwash in conjunction with standard treatments compared with standard treatments only in patient with gingivitis. The outcome of this study will be analysed using Intention To Treat (ITT) analysis. The primary outcome is the reduction in the severity of gingivitis and will be measured as a continuous variable.

The secondary outcome is the reduction of gingivitis plaque bacteria concentration and will be measured as continuous variable. Data will be analysed using Stata statistical package (STATA 11.00).

a. Initial analysis

This section will cover baseline characteristics of the study population. This will involve descriptive analysis, such as age, sex, level of education, and ethnicity. The variables which are considered to be confounders will be identified, and these include among others age, sex, education level, and ethnicity. Age will be treated as a continuous variable, while the rest of the socioeconomic variables will be measured categorically (education and ethnicity) and dichotomously (sex).

b. Primary analysis

Mean difference of severity reduction of gingivitis before and after rinsing at baseline and day 30 post-randomisation between intervention and control groups will be analysed using Student's t-test.

c. Secondary analysis

The number of gingivitis plaque bacteria will be transformed in the logarithmic scale prior to statistical analysis to approximate a normal distribution. Student's t-test statistics will be applied to the transformed variable to examine the treatment effect of each treatment group (paired student t-test) by comparing mean difference of gingivitis bacterial reduction before and after rinsing at baseline and day 30 post-randomisation, between control and intervention group.

4.9. Potential advantages of the outcome

The advantages of this study is it will provide information regarding the efficacy of *Averrhoa bilimbi* as an adjunctive therapy in gingivitis treatment.

4.10. Ethical Considerations

In order to conduct this study, ethics approval will be sought from the ethics committee at Trisakti University Dental Hospital. Participants will be informed of the procedures, benefits, and possible adverse event relating to the study.

Informed consent will be obtained in the form of a signature or thumb-print (if unable to sign) placed on the study consent form. A plain language information statement (PLIS) will be provided to the participants to ensure the participant has a clear understanding of the study procedures and methods. To ensure the confidentiality of participants, all data and information of participants will be stored in secure place and accesses only by investigator involves in this study.

5. DISCUSSION

The aim of developing this protocol is to determine the most appropriate methodology for use in the epidemiological study to investigate the efficacy of a mouthwash containing the extract *Averrhoa bilimbi* as an adjunctive therapy in gingivitis treatment. The study will be conducted in Trisakti University Dental Hospital, Jakarta.

A randomised controlled trial with double blind method is considered for selection as the most appropriate design to answer the research questions. The purpose of randomised controlled trial is to measure the effect or efficacy of the intervention on outcome. Randomisation is the method used to assign participants to different groups, control and intervention groups. Randomisation accounts for any characteristics which might influence the outcome. Randomisation will be conducted after participants eligiblility has been determined, thus, preventing potential selection bias. This study will allow participants in both control and intervention groups to receive similar standard treatment to eliminate the etiologic factor of gingivitis.

When participants rinse with mouthwash unsupervised at home, there is the potential that they do not follow the instructions as given. Therefore, the remaining mouthwash will be collected when participants visit the clinic for follow-up procedures to allow investigator to measure the residual volume of mouthwash. Lost to follow-up will be minimised through daily contact with participant cohort to ensure they follow the instructions. All potential confounding factors such as age, sex, race or ethnicity, level of education, and level of income will be identified during analysis

The potential strengths in this study are: (1) the implementation of randomisation which might reduce potential bias and confounding, (2) measurement will be assessed by single investigator which is potentially more feasible, (3) homogenous group with respect to gingivitis, (4) no painful and documented adverse events of *Averrhoa bilimbi*, and (5) the duration of this study only 30 days which reduce participants and investigator time committment.

There are also some potential limitations in this study such as: (1) participants may be differentiate between active treatment and placebo due to taste (sour versus salt taste) which may minimise the advantage of double blinding the randomised controlled trial, (2) some exclusion criteria may reduce the generalizability of this study, (3) possible bias in gingivitis bacterial measurement due to conditions or contamination which may cause the bacterial growth to reflect the confounding agents rather than the true conditions including the bacteria that is in an individual's oral mucosa.

The findings from the study will provide information regarding a mouthwash containing *Averrhoa bilimbi* extract in relation with gingivitis. This plant can easily be found and is commonly used, it can be utilize as an practical and easy to use adjunctive therapy for gingivitis. This research project is a pilot study investigating the effect of *Averrhoa bilimbi* extract in gingivitis treatment. The pre-eliminary result from this research will help future researchers to assess the association between *Averrhoa bilimbi* and gingivitis, including dosage adjustment. It is expected also that the findings in this study can help future researchers to understand the mechanisms by which *Averrhoa bilimbi* might act to reduce the severity of gingivitis, and whether *Averrhoa bilimbi* can be used daily to prevent gingivitis.

6. CONCLUSION

Gingivitis remains a worldwide health problem which affects both developing and developed countries. Poor recognition of gingivitis symptoms at the early stages of the disease, lack of education, and socio-economic factors are considered as the triggers of delay prevention of gingivitis. However, it is still a dilemma to treat gingivitis instead of preventing it, since it is better to prevent rather than to cure. The use of *Averrhoa bilimbi* extract in reducing severity of gingivitis is expected to provide more benefits to community, since it is affordable and easy to find.

This study protocol is aimed at investigating the efficacy of *Averrhoa bilimbi* extract mouthwash as an adjunctive therapy in gingivitis. The findings can be used to provide an alternative cost effective therapy in gingivitis treatment and to help future research needs.

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