



Antimicrobial activities of methanol extract of Sirih Merah (*Piper crocatum* L.) leaf

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ABSTRACT

Hand washing is a simple thing but that is very important as one as a trick prevention for infection disease. The study is implemented by using research experimental. As an independent variable is washing hands with some materials and as a dependent variable is counting the colony. The materials that used is Piper crocatum extract, hand soap with no antiseptic, and hand soap with antiseptic. Sample quantity for each material is 14 peoples. The percentage decrease of total colony numbers after washing hands with piper crocatum extract is 71.21%, with no antiseptic soap 33.68%, and 43.69% with antiseptic soap, respectively. Microbe quantity counted by before and after hand washing and analyzed by t-test paired. Hand washing used by three materials indicated statistical of microbe numeral ($p < 0,005$). Water, tissue/napkin dryer, and the behavior of hand washing can be influence the total microbe after hand washing. Decline significant mean compare of before hand washing is with use antiseptic hand soap and Piper crocatum methanolic extract.

Key words: *Piper crocatum* L. leaf, methanolic extract, bacteria colony, GC-MS, FTIR

INTRODUCTION

Indonesia is a country which rich sources of novel biological active secondary metabolites. Many researches showed that some of the Indonesia Natural products produced by plants and animals are sources of of bioactive compounds potential to be developed as medicine. Some of them are mahoni (*Swietenia mahagoni* Jacq, Kumis kucing (*Orthosiphon spicatus* BBS), Mengkudu (*Morinda citrifolia* L) and sirih merah (*Piper crocatum* L).

The plant is a traditionally used by local people at North Sumatera and West Sumatera region. to cure various diseases such as diabetes, hepatitis, kidney failure, stroke, hypertension, candidiasis etc [1-5]. However, there are only limited studies on bioactivity.

One of medicinal plants commonly used by people is betel plant. Betel plant has been known as an antiseptic since 600 BC. The types of betel generally used as medicine in Indonesia are green betel (*Piper betel*) and black betel. However, there is another type gaining popularity i.e. red betel. Red betel is the type of betel often used as ornamental plant until 1990s. Sirih merah (*Piper crocatum* L) is an ornamental plant with medicinal properties. Infusion of sirih merah is traditionally used as antiseptic, anti-diabetic, anticancer and to cure the infection diseases. The major categories of plant derived compounds that have medicinal properties are terpenoids, flavonoids, and Alkaloids[6]. *Piper crocatum* Ruiz and Pav. is traditionally used by Indonesians for treating various diseases, including breast cancer and as antiseptic, however there is no scientific evidence currently available.

The present work described the used of Sirih merah ((*Piper crocatum* L) as an antiseptic of hand washing.

EXPERIMENTAL SECTION

2.1. *Piper crocatum* L. leaf Herbarium Test

Herbarium test was performed to identify and ensure the family and species of red betel plant to be examined based on its morphological characteristics. The result showed that the red betel used belonged to Piperaceae family and of *Piper crocatum* species.

2.2. Plant extract

The leaves of *Piper crocatum* L were collected from the house garden in Padang, West Sumatera province. The plant was identified at herbarium Biology Department of Faculty of Mathematics and Natural Sciences, Andalas University, Padang. The air-dried were ground to a powdery consistency. The powder was soaked in 2 litres of 96% methanol dilution for 3 days, changing the dilution every 24 hours, and then strained using filter paper. The filtrate resulted from the straining was vaporized using rotary vacuum evaporator at 40°C to get a concentrated extract of *Piper crocatum* L. This extract was then divided into several testing concentrations. The specimen was deposited and the extraction was conducted at the Laboratory of Physiology, Faculty of Medicine, the University of Prima Indonesia with 2 repetitions. The design of this research consists of hand washing using *Piper crocatum* extract, soap with and without *Piper crocatum* L. methanol extract.

2.3. Equipment and Chemicals

FT-IR obtained from Hitachi, Japan, GC-MS from Jaso, Japan. All chemicals used were obtained from Sigma unless otherwise noted.

2.4. Antimicrobial Activity Test

Antimicrobial activity [9] of hand soaking with and without methanol extract of *Piper crocatum* L. leaf was determined by using modified disk method. Purified single colony from isolate was transferred into sterilized MRS broth (Merck), incubated at 37°C for 48 hours. The culture was centrifuged at 10.000 rpm, 4°C, for 20 minutes. A number of colony was then calculated.

RESULTS AND DISCUSSION

3.1. Identification and Extraction of *Piper crocatum* L. leaf

The identification result of red betel leaves based on the herbarium test performed at the Herbarium Laboratory of The Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University. showed that the fresh red betel leaves collected from housing garden were from red betel plant (*Piper crocatum*). 400 g of fresh leaves was air dried to produce 150 g of dried red betel leaves. After being macerated in 2 litres of 96% methanolic dilution for 3 days, it produced *Piper crocatum* L. leaf methanolic extract of blackish green color. The extract was then used for hand washing.

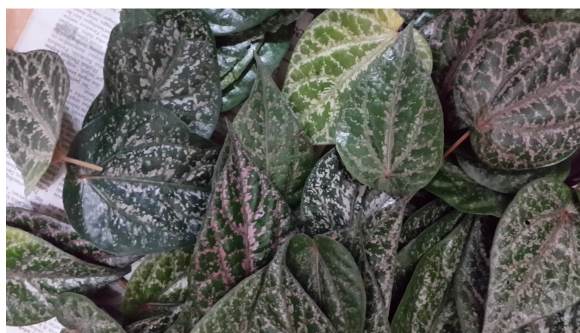


Fig. 1. *Piper crocatum* L. (Sirih merah) leaf

3.2. Phytochemical Screening

The result of the *Piper crocatum* L. leaf methanolic extracts phytochemical screening showed the presence of alkaloid, flavonoid, saponin, triterpenoid, and tannin compounds. Flavonoid contained in leaves comprises flavonol, flavanon, isoflavon, auron, catechin, antocyanidines dan chalcones [6-8, 10]. Other active substances such as tannin, saponin and alkaloid were also found in the methanolic extract of leaf. The difference can be caused by a lot of factors such as organ differences, cells, age of plants, seasons, and geographical location of the plants.

The essential oils were isolated by conventional hydrodistillation of the fresh leaves of *Piper crocatum*. It gave oil in 0.26 % yield (w/w) on a fresh weight basis. GC-MS analysis of the oil resulted in the identification of total of 35 components. The major identified components of *Piper crocatum* leaf oil were sesquisabinene hydrate (22.83 %), 8-

epi- β -bisabolol (17.24 %), γ -curcumene (11.16 %), anymol (3.90 %), α -cedrene (3.70%), and trans- β -Farnesene (3.61%).

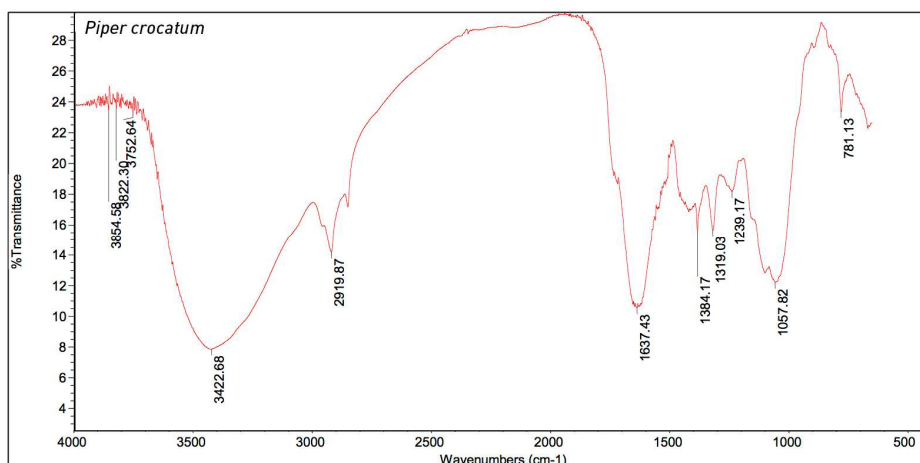


Fig. 2. FT-IR *Piper crocatum* L. leaf

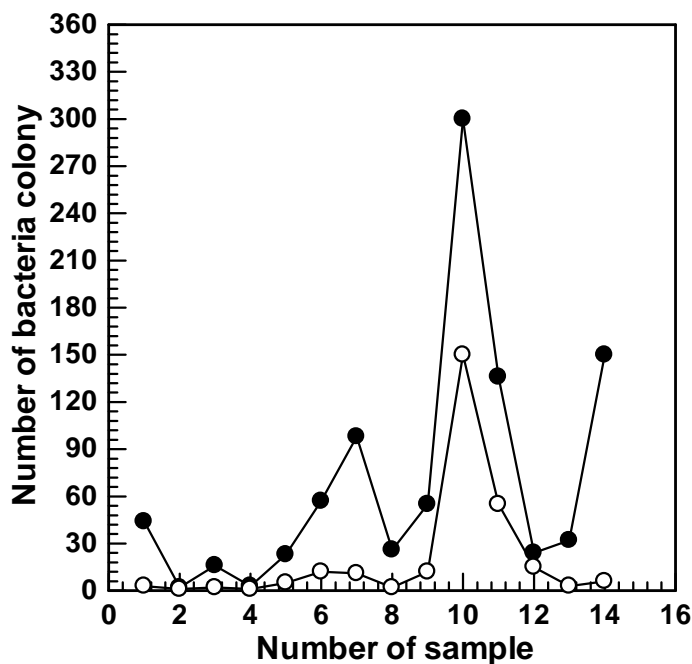


Fig. 3. Decreasing number of bacteria colony before and after hand washing with *Piper crocatum* methanolic extract. (●) before hand washing and (○) after hand washing

The active ingredients of *Piper crocatum* oil leaves were primarily reported as a class of allyl-benzene compounds where particular emphasis has been placed on chavibetol (betel-phenol; 3-hydroxy-4-methoxyallylben-zene). It also contains chavicol (p-allyl-phenol; 4-allyl-phenol), estragole (p-allyl-anisole; 4-methoxy-allylbenzene), eugenol (allylguaiacol; 4-hydroxy-3-methoxy-allylbenzene; 2-methoxy-4-allyl-phenol), methyl eugenol (eugenol methyl ether; 3,4-dimethoxy-allylbenzene), and hydroxycatechol (2,4-dihydroxy-allylbenzene). Allylbenzene compounds such as chavicol, estragol, eugenol, and methyl eugenol which were well known for their antibiotics properties was not found in *P. crocatum* essential oils(10). However, sesquiterpene γ -curcumene (11.16%) and α -curcumene (0.56%) which contributed to the aromatic properties of *Curcuma* sp essential oil, were actually also found in *P. crocatum* essential oil.

3.3. Fourier Transform Infra Red (FT-IR) Spectroscopy Analysis

FT-IR spectra indicated that the functional group present at *Piper crocatum L.* eaves was carbonyl, hydroxile, carboxylate and amine. The functional group present at *Piper crocatum L.* leaf was similar with the compounds resulted from GC-MS analysis. The results are shown in Fig. 2.

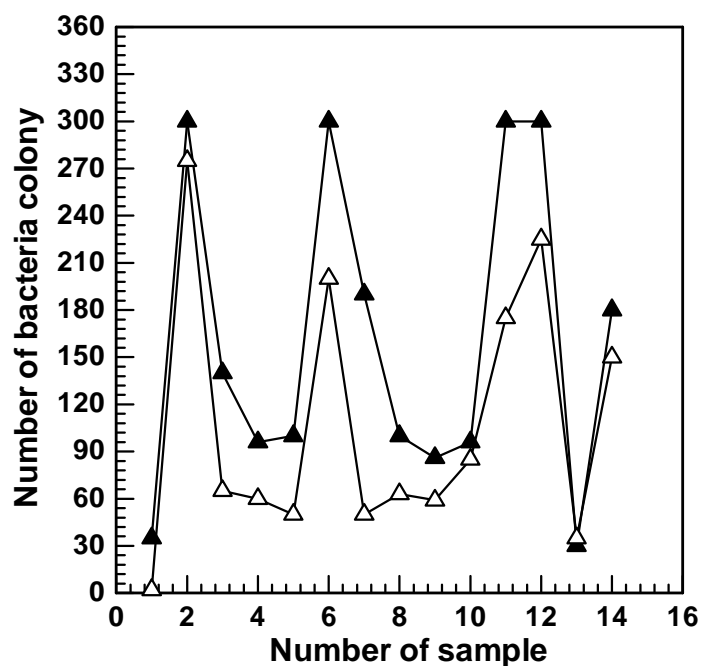


Fig. 4. Decreasing number of bacteria colony before and after hand washing with soap with and without antiseptic. (▲) before hand washing and (△) after hand washing

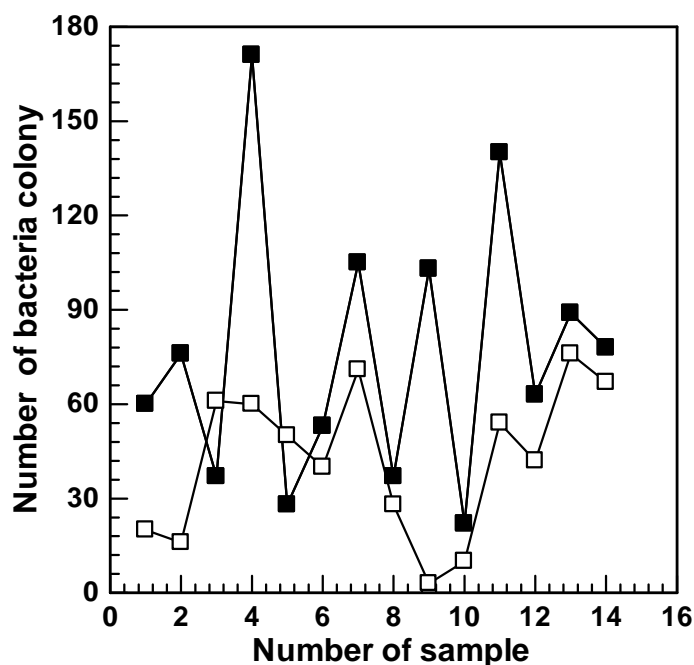


Fig. 5. Decreasing number of bacteria colony before and after hand washing with soap contain antiseptic. (■) before hand washing and (□) after hand washing

3.4. Analysis of colony bacteria

Number of colony bacteria was calculated based on the hand treatment with and without hand washing by using methanol extract of *Piper crocatum L.*

As an independent variable is washing hands with some materials and as a dependent variable is counting the colony. The materials that used is *Piper crocatum* extract, hand soap with no antiseptic, and hand soap with antiseptic. Sample quantity for each material is 14 peoples. The percentage decrease of total colony numbers after washing hands with *piper crocatum* extract is 71.21%, (Fig. 3), with no antiseptic soap 33.68% (Fig. 4), and 43.69% (Fig. 5) with antiseptic soap, respectively. Microbe quantity counted by before and after hand washing and analyzed by t-test paired. Hand washing used by three materials indicated statistical of microbe numeral ($p < 0,005$). Water, tissue/napkin dryer, and the behavior of hand washing can be influence the total microbe after hand washing. Decline significant mean compare of before hand washing is with use antiseptic hand soap and *Piper crocatum* methanolic extract. The colored of bacteria colony indicated that the bacteria in the form of coccus (Fig. 6A) and basil (Fig. 6B)

The research shows that it is necessary to identify the species of bacteria whether it is pathogen or not.

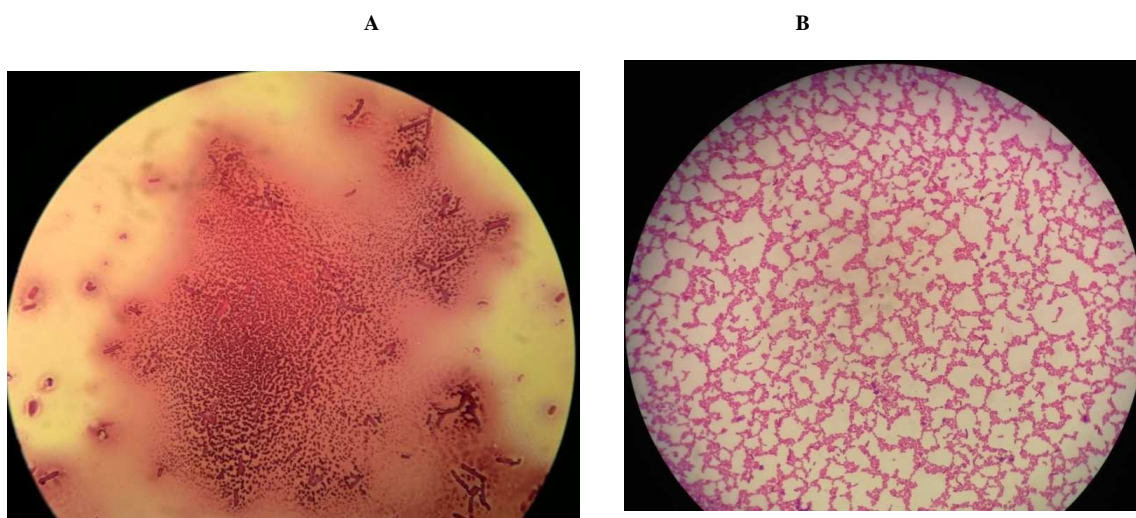


Fig. 6. Colored bacteria colony detected at hand sample in form of coccus (A) and basil (B)

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