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Topical Antibiotic Optimization of Wijayakusuma Plant (*Epiphyllum oxypetalum*) as Mosquito Repellent Therapy Oil

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Abstract

Wijayakusuma plants contain various active compounds that are beneficial to health, such as alkaloids, triterpenoids, and saponins. This research aims to optimize topical antibiotic products from *Wijayakusuma* plant that were synergized with *Sambung Nyawa, Sambang Darah, Bratawali, Sambilata*, and Tobacco leaves into a therapeutic oil for a mosquito repellent. The topical antibiotics were made by maceration method by soaking *wijayakusuma, sambung nyawa, sambilata, bratawali, sambang getih*, and tobacco leaves using VCO then tested by viscosity test, antibacterial test, phytochemical screening, antioxidant test, and efficacy test. Data were analyzed with descriptive statistics. The results showed that viscosity measurement with Oswald 20^{o}C showed 22.56 cP. Antioxidant analysis used the % DPPH inhibition method with a sample of 50,000 ppm was 51.6%. Antibacterial activity against Bacillus subtilis (gram-positive) was 18.8 mm (strong), and against Escherichia coli (gram-negative) was 10.4 mm (strong). The equivalent flavonoids as topical antibiotics contained 15.51 mg QE/ml, phenolics 8.11 mg GAE/ml, and tannins 11.30 mg TAE/ml. Mosquitoes stumbled and flew away when exposed to this topical antibiotic. This topical antibiotic has good antioxidant content for body care, antibacterial and aromatherapy that repels mosquitoes, and is suitable for external massage oil treatment.

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Introduction

Wijayakusuma (*Epiphyllum oxypetalum*) is a sacred plant for the Javanese people. Cilacap Regency government even declared *Wijayakusuma* flower as the icon of the Cilacap Regency. *Wijayakusuma* plant (*Epiphyllum anguliger*) is an ornamental plant that originated in South America. This plant has a thin, long, curved stem like a cactus, with small leaves growing along the stem. The color of the leaves is dark green, and they have a unique zig-zag shape. This plant also has beautiful white flowers with pink lines. The *wijayakusuma* plant belongs to the cactus family or Cactaceae. Some studies have shown that the *wijayakusuma* plant contains active compounds, such as flavonoids, phenols, and amino acids, which provide antioxidant, anti-inflammatory, and anticancer properties. A study by Kusumawati et al. (2019) mentioned that there are antioxidant and anti-inflammatory activities in the ethanolic extract of the *wijayakusuma* plant leaves. The result of this study showed that the extract of *Wijayakusuma* had high antioxidant and anti-inflammatory activities due to the presence of flavonoid and phenolic compounds. Therefore, the *Wijayakusuma* plant can potentially develop into an active ingredient in the pharmaceutical industry.

Some herbal therapists in Maos District developed topical antibiotics from the *Wijayakusuma* plant extracted by soaking them in *klentik* (coconut oil) or lemongrass oil. In herbal medicine theory, it is not recommend to use a single herb. Therefore, the therapists synergize with plants such as *Sambung Nyawa*, *Sambang Darah*, *Sambilata*, and tobacco. In their treatment practice, therapists use it as a topical antibiotic to treat wounds, itches, and acnes. However, this *Wijayakusuma* plant as the topical antibiotic ingredient can still be improved in terms of function and usefulness by formulating them into herbal therapy oil products, such as massage oil and body scrub, which can also repel mosquitoes using a formulation method that meets modern pharmaceutical standards. This study aims to optimize the formulation of topical antibiotics from *Wijayakusuma* plant synergized with other ingredients that comply with current pharmaceutical standards, as well as to test the effectiveness of its use as a massage oil and scrub to repel mosquitoes.

Methods

This research was conducted from April to September 2022 in the Biology Laboratory of SMA Negeri 1 Maos, Cilacap Regency. The laboratory tests were conducted at the FSM UKSW Surakarta's Chemistry Laboratory. Data collection was carried out by running a series of experiments procedures: (1) Measurement of fluid viscosity using viscosity tests (Nowrouzi et al., 2020); (2) Measurement of antioxidant content using the % inhibition of the DPPH method (Pulung et al., 2016); (3) Antibacterial activity test with gram-positive bacteria *Bacillus subtilis* and gram-negative bacteria *Escherichia coli* (Rahmadi et al., 2013); (4) Measurement of biochemical compounds using Spectro preparation to measure the content of flavonoids, tannins, and phenolates (Julianto, 2019); (5) Mosquito repellent test by exposing the topical antibiotic solution to mosquitoes. (Boesri et al., 2015).

Data analysis followed the steps: (1) Analyze the fluid viscosity compared to water at a temperature of 20°C with the unit of centipoise (cP); (2) Analyse the antioxidant content by comparing samples of 0 ppm, 5,000 ppm, 10,000 ppm, and 50,000 ppm, (3) Analyze the antibacterial activity by comparing the distance between the antibiotic and the growth of gram-positive and gram-negative bacteria in agar plates, (4) Analysis of flavonoid, tannin, and phenolate content using Spectro preparation, (5) Mosquito repellent activity test by observing the behavior of mosquitoes when exposed to the topical antibiotic.

Results

Commonly, the community produces topical antibiotics for non-oral use, such as massage or topical oil, by soaking herbal ingredients in coconut oil. Although this traditional method can produce topical antibiotics, it could be more optimal regarding active compound content. Coconut oil production using heating method has a low quality, causing it become rancid quickly. Moreover, the herbal ingredients soaked in coconut oil are mostly not dried, which can accelerate rancidity and decrease the product quality. Therefore it is necessary to improve the use of appropriate maceration methods with indicators in the form of phytochemical screening results, antioxidant compounds, and antibacterial activity.

The traditional technique of maceration (soaking) was good. However, it still needed improvement in terms of the medium used for soaking herbs and the dryness level of the herbal materials used. For this purpose, the following steps to improve the traditional method need to be done: (1) substitute *klentik* oil with VCO (Virgin Coconut Oil); (2) handling the herbal materials before maceration by chopping the herbal materials into small pieces to maximize the maceration process than drying the herbal materials until the moisture level content below 10% to produce a better quality product that did not make it rancid quickly.

With the improvement of this manufacturing technique, the result of the antibiotic product will have a better quality and it will more durable.

Table 1. Laboratory Test Analysis Results

Parameter	Result	Unit
Viscosity	22.56	Cp
% Antioxidant Inhibition on 50000 ppm antibiotic	51.6	%
Antibacterial Activity Gram-positive (*B. subtilis*)	18.8 (strong)	Mm
Antibacterial Activity Gram-negative (*E. coli*)	10.4 (strong)	Mm
Total Flavonoid Content	15.51	mg QE/ml
Total Phenol Content	8.11	mg GAE/ml
Total Tannin Content	11.30	mg TAE/ml

The detailed data testing as follow:

Table 2. Viscosity Test Analysis

a. Density: Measurement results data at Chemistry Laboratory

Sample	Mass (g)				r
	I	II	III	$\bar{x}$	(g/ml)
Topical antibiotic (1 ml)	0.9587	0.9584	0.9586	0.9586	0.9586

b. Oswald Viscosity Measurement at 20°C: topical antibiotic sample

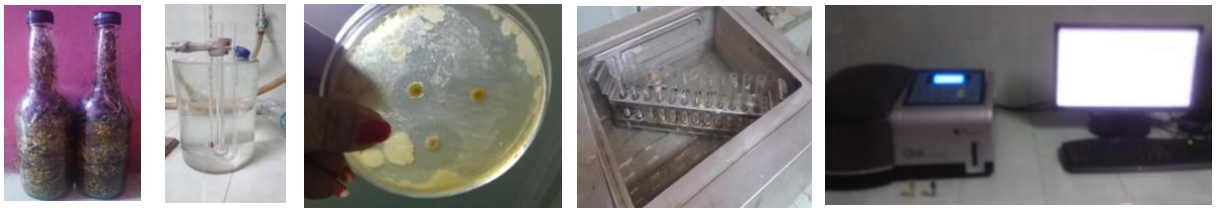
Sample	t(s)				r	H
	I	II	III	$\bar{x}$	(g/ml)	(cP)
Destilled water	1.62	1.60	1.55	1.59	0.9980	1.002
Topical antibiotic (1 ml)	37.15	37.47	37.20	37.27	0.9586	22.561

Antioxidant measurement analysis was carried out using the % inhibition DPPH method:

Table 3. Percentage of Inhibition DPPH Analysis

Sample	A_{517nm} dpph			Average	% Inhibition
	I	II	III		
Blank	0.686	0.686	0.686	**0.686**	**0.00**
Antibiotic 5000 ppm	0.640	0.640	0.640	**0.640**	**6.71**
Antibiotic 10000 ppm	0.570	0.570	0.570	**0.570**	**16.91**
Antibiotic 50000 ppm	0.332	0.332	0.332	**0.332**	**51.60**

The antibacterial activity test was carried out against Gram-positive bacteria (B. subtilis) and Gram-negative bacteria (E. coli) with the result that this oil-type topical antibiotic has strong antibacterial properties. Then, the flavonoid, tannin, and phenol content were measured using Spectro preparation. These compounds have been proven effective against the dangers of free radicals (Lumingkewas et al., 2014).



(a) (b) (c) (d) (e)

Figure 1. (a) Topical Antibiotic Production Process; (b) Viscosity Test; (c) Antibacterial Activity Test; (d) Analysis of Flavonoid, Tannin, and Phenol Content; (e) Antioxidant Analysis

Discussion

Topical antibiotics are ointments or massage oils that can be used as therapeutic agents to help cure various diseases. According to Suhariyanto (2018), antibiotics are substances produced by or derived from fungi, bacteria, and other specific organisms that can damage or inhibit the growth of other microorganisms. According to Bhatt et al. (2014), a topical antibiotic can be recommended if it has antimicrobial and immunomodulatory activities. Topical preparations are formulations used on the skin to produce a local effect, such as lotions, ointments, and creams. Therefore, topical antibiotics are the most commonly prescribed medicine by dermatologists to treat mild to moderate *acne vulgaris*, and they are also adjunctive therapy with oral medications.

There are two types of acne treatment; topical treatment, which is directly applied to the affected area to produce a local effect, and oral treatment, which is take to treat acne through systemic pathways (Madelina et al., 2018). Routine use of topical and oral antibiotics can lead to antibiotic resistance. Therefore, The Global Alliance to Improve Outcomes in Acne recommends that topical and oral antibiotics not be combined as monotherapy. In the Guidelines for Pharmaceutical Services for Antibiotic Therapy from the Ministry of Health (2011), it is stated that the usage of topical antibiotics should be limited to use only

on the eyes and ears because they can cause antibiotic resistance and hypersensitivity. If topical antibiotics are necessary, choose antibiotics not absorbed through the skin (not systemic antibiotics), for example, Mupirocin.

One of the topical antibiotic products is a synergistic herbal oil produced by PT. Herba Penawar Alwahida Indonesia (2013). Traditionally, topical antibiotic used as a massage oil and ointment to help relieve muscle aches, joint pain, and bruises. Its contents include Virgin Coconut Oil, *Kencur* Rhizome (*Kaemferia galanga*), *Bratawali* (*Tinosporae caulis*), Cinnamon Bark (*Cinnamomi Burmannii* Cortex), *Sambiloto* (*Andrographidis paniculatae* Herba.), *Oleum olea Europea*, *Oleum elaeis* Gineensis, and *Egenia caryophili* Flos.

The herbal therapists usually use *klentik* or traditional coconut oil as the base. Regulation No. 32 of 2019 by the Food and Drug Administration stated that External Drug Liquids are Traditional Medicine preparations in the form of oil, solution, suspension, or emulsion, made from simplistic and/or extracts and used as an external medicine. Meanwhile, the process of making coconut oil (*klentik)*, according to the Decree of the Minister of Health of the Republic of Indonesia No. HK.01.07/MENKES/187/2017 is by grating one ripe coconut, adding four glasses of water, squeezing it, straining it, then cooking it until it becomes oil, which is then apply to the affected area. This oil was made by heating coconut milk water 90-120°C for approximately 4 hours. The result is clear golden yellow coconut oil.

The poor quality of coconut oil has high peroxide and free fatty acid numbers, a stink odor, a yellowish-brown color, and becomes rancid quickly in less than two months (Hari et al., 2017), which is different from virgin coconut oil (VCO). VCO can be made without heating or at a specific temperature. VCO contains a relatively high amount of medium-chain fatty acids (MCFA). The most abundant MCFA in VCO is lauric acid. Lauric acid in VCO is antiviral, antibacterial, and antiprotozoal. Research on Human Immunodeficiency Virus (HIV) patients has shown that giving VCO positively affects HIV patients. Lauric acid is proven to be a natural antibiotic for infected skin with *Propionibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermis* in vitro and in vivo. Lauric acid also has high antioxidant activity. Polyphenols, one of the bioactive components in VCO, have been proven to inhibit arthritis (Karouw et al., 2014). Therefore, topical antibiotics should preferably use VCO as the base.

The active ingredients of this topical antibiotic were the leaves and stems of the *Wijayakusuma* plant, which can be used to treat inflammation, wounds, abscess. It is also can be for internal use to treat cough, anti-inflammatory, and others (Artini et al.,2018). The compounds contained in the petroleum ether extract of the *Wijayakusuma* plant are alkaloids, triterpenoids, and saponins. These metabolites can cause blood hemolysis, making them toxic and bioactive as antifeedants. Other phytochemical contents of the *Wijayakusuma* plant include citric acid, which is effective to stop bleeding (hemostatic), relieving cough, and phlegm, as well as having anti-inflammatory properties (Hasanuddin, 2015)

The complementary ingredients of the topical antibiotic are Sambung Nyawa (Gynura procumbens), Sambang Getih (Hemigraphis bicolor Boerl.), Bratawali (Tinospora crispa (L.) Miers), Sambilata (Andrographis paniculata Nees), and tobacco leaves. The Sambung Nyawa (Gynura procumbens) contains chemical compounds such as flavonoids, unsaturated sterols, triterpenoids, polyphenols, saponins, steroids, chlorogenic acid, caffeic acid, vanillic acid, p-coumaric acid, p-hydroxybenzoic acid, and essential oils that, according to Fadli (2015), have an effect on inhibiting cancer cell growth. In addition, the chemical compounds found in the Sambung Nyawa can be use as antifungal, antiamebic, larvicidal, antimicrobial, antioxidant, anti-allergic, and analgesic agents. Moreover, according to Putri & Tjitraresmi (2017), Sambung Nyawa can also be used as an antidiabetic, antiproliferative, antihypertensive, antibacterial, organ protector, sexual function enhancer, antioxidant, and can be used for hypoglycemic identification. Even the extract of Sambung Nyawa leaves, according to Sinaga et al. (2017), is effective as an antioxidant in coconut oil due to flavonoids, which function as natural antioxidants.

Sambang Getih plant (*Hemigraphis bicolor* Boerl.) contains various active compounds such as flavonoids, alkaloids, saponins, and polyphenols with pharmacological activities such as anti-inflammatory, antidiabetic, anticancer, and as antioxidants. A study conducted by Suriyavathana et al. (2020) showed that the extract of *Hemigraphis bicolor* Boerl. contains flavonoids and phenolics that have intense antioxidant activity. In addition, the section of *Hemigraphis bicolor* Boerl. leaves also contain alkaloids and saponins as potentially active ingredients in drug development.

The stem of *Bratawali* plant (*Tinospora crispa* (L.) Miers) contains various compounds, such as alkaloids, flavonoids, saponins, tannins, and polyphenols, that have different pharmacological effects, including anti-inflammatory, antidiabetic, anticancer, and immunomodulatory effects. Evaluation of the antioxidant and anti-inflammatory activities of ethanol extract of *Bratawali* stem by Wibowo et al. (2019) showed that the extract has intense antioxidant activity, possibly due to its flavonoid and polyphenol content. The ethanol extract of *Bratawali* stem also has significant anti-inflammatory activity, possibly due to the presence of alkaloids and saponins.

Bratawali often uses as a remedy for fever, cholera, rheumatism, jaundice, and type II diabetes. The active ingredients in the roots, stems, leaves, fruits, and flowers of *Bratawali* can also control plant pests, including alkaloids, tannins, saponins, glycosides, terpenoids, and flavonoids and their derivatives. In addition to being toxic to insects, *Bratawali* plant also has antifungal, nematicidal, and anti-molluscan properties. The stem of *Bratawali* can be used to control various insects such as mites, *Spodoptera exigua*, *Nephotettix* spp, *Nilaparvata lugens*, *Plutella xylostella*, *Phyliotera sinuata* Ateph, *Scirtothrips dorsalis* Hood, *Phyllocnistis citrella* Stainton and *Culex quinquefasciatus* mosquito larvae with effectiveness values above 50% and production efficiency ratios lower than synthetic pesticides. Moreover, *Bratawali* can also be used as a component in organic pesticide mixtures (Wiratno et al., 2019). The *Bratawali* plant contains an active compound called tinocrisposide, which is an antimalarial. Tinocrisposide works in the erythrocyte phase by inhibiting parasite growth in erythrocytes. Bratawali stem extract can be used as an alternative treatment for malaria patients (Malik, 2015).

Sambilata (*Andrographis paniculata* Nees) is a medicinal plant with various benefits, one of which as an antibacterial agent because according to Sikumalay et al. (2016), it contains the main active component, andrographolide. Sambilata has antimicrobial activity against nine bacteria: Salmonella *typhimurium*, *Escherichia coli*, *Shigella sonnei*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia*, *Streptococcus pyogenes*, *Legionella pneumophila*, and *Bordetella pertussi*. According to Royani et al. (2014), andrographolide has been proven to have activities such as hepatoprotective, cardiovascular, hypoglycemic, psycho-pharmacological, anti-fertility, antibacterial, immunostimulant, antipyretic, antidiarrhoeal, anti-inflammatory, antimalarial, antivenom, and antihepatotoxic. Andrographolide is most accumulated in the leaves of the sambilata plant (2.39%) while the lowest amount is found in the seeds. Sambilata extract, according to Nugroho et al. (2016), also has the potential as a disinfectant against *Leptospira* sp. bacteria at a minimum dose of 1.56%.

Tobacco leaves enhance their mosquito repellent properties because they contain nicotine, the primary alkaloid compound that acts as an insecticide. Nicotine can be a potent nerve poison for insects and is used as a raw material for various insecticides. The higher the concentration of nicotine sulfate, the faster the effect on insect death. Tobacco extract has larvicidal activity against *Aedes aegypti* mosquitoes (Aji et al. 2015). The larvicidal activity of tobacco leaf extract is not directly proportional to the nicotine content in the extract (Handayani et al., 2018). The study by Hidayati et al. (2018) showed that tobacco leaf extract has larvicidal solid effects against *Aedes aegypti* and *Aedes albopictus* mosquitoes, with LC_{50} values of 0.11% and 0.20%, respectively. Meanwhile, a study by Kusumawati et al. (2020) showed that tobacco leaf extract could kill *Aedes aegypti* mosquitoes with an LC_{50} value of 0.132%. The active compounds in the section are believe to come from nicotine, chlorogenic acid, ferulic acid, anabasine, and cinnamic acid content.

The sinergy of *Wijayakusuma, Sambung Nyawa, Sambang Getih, Bratawali, Sambilata*, and Tobacco leaves in VCO based, created a topical antibiotic preparation with excellent quality. It could be sprayed or applied to areas of the body that experience itching, soreness, pain, boils, acne, mosquito bites, and similar conditions. Before use on humans, this topical antibiotic product was tested on rabbits by applying it to their skin thrice daily. The results showed no irritation or redness.

Figure 2. Topical Antibiotic Products

Furthermore, an anti-mosquito test was conducted by exposing the topical antibiotic to the mosquitoes. The product's scent made the mosquitoes look dizzy and unstable, and the tobacco scent has prove to have bioinsecticidal effects that prevent mosquitoes to stay longer. Thus, users of the topical antibiotic can be free from mosquitoes bite. The repellent test using the formula: DR = $\frac{k-p}{k}$ x 100%, where k is the negative control, and p is the number of mosquitoes that landed on the sample given treatment. The result was 100%. It means that it have good repellent power.

Conclusion

Topical antibiotic make using the maceration method by soaking dried *Wijayakusuma, Sambung Nyawa, Sambilata, Bratawali, Sambang darah*, and tobacco leaves with a moisture content of less than 10% using VCO. Viscosity measurement with Oswald at 20^{o}C showed a value of 22.56 cP. Meanwhile, antioxidant analysis using the % inhibition DPPH method with a 50,000 ppm sample yielded 51.6%. The antibacterial activity against *Bacillus subtilis* (gram-positive) was 18.8 mm (strong) and against *Escherichia coli* (gram-negative) was 10.4 mm (strong). The flavonoid content in the topical antibiotic was 15.51 mg QE/ml, phenol was 8.11 mg GAE/ml, and tannin was 11.30 mg TAE/ml. Based on the testing results conducted on mosquitoes exposed to this topical antibiotic, the mosquitoes acted dizzy and flew away. This topical antibiotic is 100% success for mosquitoes repellent. Therefore, this topical antibiotic has a good antioxidant content for body care, antibacterial, and aromatherapy properties that mosquitoes do not like, and potentially for external oils medicine.

Author Contributions

Conceptualization, Agus Darwanto and Kis Rindiana Subroto; methodology, Agus Darwanto; software, Agus Darwanto.; validation, Agus Darwanto, Kis Rindiana Subroto and Novita Dian Susilowati; formal analysis, Agus Darwanto; investigation, Kis Rindiana Subroto and Novita Dian Susilowati; resources, Kis Rindiana Subroto dan Novita Dian Susilowati; data curation, Agus Darwanto; writing—original draft preparation, Kis Rindiana Subroto and Novita Dian Susilowati; writing—review and editing, Agus Darwanto; visualization, Kis Rindiana Subroto and Novita Dian Susilowati; supervision, Agus Darwanto.; project administration, Kis Rindiana Subroto; funding acquisition, Novita Dian Susilowati. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest:

The authors declare no conflict of interest. The funders had no role in the study's design; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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