

POTENTIAL COMBINATION OF VIRGIN COCONUT OIL (VCO) AND AZADIRACHTA INDICA EXTRACT FOR ACNE CONTROL: A COMPOSITION PHYTOCHEMICAL AND IN VIVO ANTIBACTERIAL EVALUATION

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Abstract

Background: Treatment of acne can be done with topical and systematic therapy. The combination of VCO (Virgin Coconut Oil) and Intaran (Azadirachta indica) leaf extract (VCO-I) has the ability to inhibit bacteria in vitro. Thus, further testing of its activity in vivo is necessary. **Aims:** The objective is to assess the phytochemical composition and the effect of different concentrations of VCO-I on P. acnes-induced inflammation. **Method:** This study has a posttest-only control group design and is experimental. Rats in the experimental group were smeared with VCO-I at concentrations of 0% (P0), 5% (P1), 10% (P2), and 20% (P3); clindamycin (Cindala®) was used as the positive control (K+); and distilled water was used as the negative control (K-). **The results:** VCO-I contained flavonoids, tannins, and alkaloids. The level increases as the mixture's concentration increases. Each VCO-I treatment was able to treat wounds or lesions that appeared due to the induction of P.Acne bacteria. There are differences in each treatment for healing the lesions. Based on the Post Hoc Bonferroni test P2 and P3 were not significantly different from K+, and K0 was significantly different from P0. **Conclusion:** The addition of the extract can increase the activity of speeding up the healing of lesions, and the effective mixture is at P2 and P3.

Keywords: bacterial patterns, antibiotic susceptibility, Tabanan General Hospital

1. Introduction

Biological exposure to the environment can cause skin disorders such as acne known as acne vulgaris (AV). Acne is a chronic infection of the skin (1). As a result of this infection appears on the skin of the face, neck, chest, and back. Human skin disease is characterized by the presence of pimples, red scales, whiteheads, papules, and nodules. The main cause of this infection is the anaerobic

bacterium Propionibacterium acnes (2). These bacteria are involved in the development of inflammatory acne by activating the complement and metabolism of sebaceous triglycerides into fatty acids by irritation of the follicular wall and around the dermis (3).

Acne treatment can be done with topical and systematic therapy. Topical therapy is carried out by administering antibiotics, comedolytic agents, and anti-inflammatory

drugs (1). Excessive use of drugs for a long time can lead to an increase in bacterial resistance. This drug has limitations related to toxicity and side effects as well, such as dry skin, headaches, and nausea (4). Due to the presence of bacterial resistance from drugs, other alternatives are needed that can utilize natural ingredients so that side effects can be reduced (5). Several natural ingredients that have been studied have the potential in vitro to inhibit the growth of *P. acnes*, namely supplementation of virgin coconut oil (VCO) with intaran leaf extract (*Azadirachta indica*) (6).

VCO has been shown to exhibit anti-inflammatory activity in acute inflammation. Research has proven that VCO exhibits antioxidant, anti-inflammatory, antibacterial, wound healing, and moisturizing properties (7). Intaran leaves contain active polyphenolic compounds that have antibacterial, antioxidant, and anti-inflammatory activities (8). The leaf extract has antibacterial activity on health-related bacteria such as *P. aeruginosa*, *P. mirabilis*, *S. aureus*, *B. cereus*, and *E. Coli* (9).

VCO supplementation with intaran leaf extract qualitatively at a positive 5% concentration contained tannins, flavonoids, terpenoids, saponins, and alkaloids. In vitro, the average diameter of the VCO inhibition zone supplemented with intaran leaf extract at concentrations of 5%, 10%, 15%, 20% and 25% on the growth of *P. acnes* was 9.4 mm; 18.3 mm; 9.9 mm; 12.6mm; and 9.9 mm.

Concentrations of 5%, 15%, and 25% of intaran leaf extract were categorized as moderate, 10% and 20% of intaran leaf extract concentrations were categorized as strong. VCO supplementation with intaran leaf extract aims to provide more benefits from these two products. Apart from being an antibacterial, VCO can also maintain skin health and moisture. So that the combination of the two provides antibacterial and skin care power (6). In this study, quantitative phytochemical tests and in vivo tests have not been carried out. Therefore, further in vivo tests are needed to see the anti-acne potential at concentrations that have been effective. The urgency of this research is to produce a product from VCO supplementation with intaran leaf extract which is proven in vivo as an anti-acne. This product is expected to be a VCO product development that has been trained at KWT Taksu Tridatu into an applied product for acne treatment therapy. In addition, it can be used as a study on supplementation of other ingredients with VCO in health.

2. Method

This research is included in the type of experimental research. Experimental research is research conducted to determine the consequences of a treatment given intentionally by researchers. The experimental method is a research method used to find the effect of certain treatments on others under controlled conditions. The type of research

design used is experimental research in the category of posttest-only control group design. The research samples were VCO supplemented with intaran leaf extract with various concentrations. The experimental group was mice that were smeared with VCO and supplemented with intaran leaf extract with concentrations of 0% (P0), 5% (P1), 10% (P2), and 20% (P3). The positive control group (K+) were rats that were given Clindamycin (Cindala ®). The negative control (K-) was the rat that was smeared with distilled water. P0 is a treatment involving only virgin coconut oil without the addition of intaran leaf extract. This is conducted to observe if VCO alone affects acne healing and to provide information about its influence when combined with the extract. Meanwhile, the negative control is a control treated solely with distilled water. The total number of mice used in the study was 24 mice. Treatments for mice included those treated with clindamycin (K+), totaling 4 mice; those treated with distilled water (K-), totaling 4 mice; and each treatment with P0, P1, P2, and P3, each using 4 mice.

Data were collected through laboratory tests to determine levels of phytochemicals (flavonoids, tannins, alkaloids, and saponins), and inflammation in rats was performed by inducing 0.02 ml *P. acne* colonies intradermally in rat ears that had been cleaned and left for 2 x 24 hours until inflammation occurs. The stages of the research included the preparation of intaran leaf extract, the

preparation of the supplementation mixture, the phytochemical test, the pH test and the in vivo test in terms of the degree of inflammation.

Determination of total flavonoids using a spectrophotometer with the $AlCl_3$ method (10). The analysis of the total tannin extract was conducted utilizing the Folin-Denis method (11). The alkaloid test was performed using the Mayer method and saponin screening by Frothing Test (12).

In vivo test

In the in vivo test, each of the VCO that had been supplemented with the extract, as well as the control was applied 2 times a day in the morning and evening at the site of inflammation in mice. Observations were made on days 5, 10, and 15. Assessment was based on the degree of severity of the lesion, namely a score of 0 for a skin condition where there were no lesions, a score of 1 for a comedo (a pore blockage without inflammation), a score of 2 for a papule (a solid mass that protruding above the skin measuring up to 0.5 mm and red and without filling), score 3 for pustules (lesions that are inflamed and filled with pus/pus) (13). In vivo test data analysis was carried out using the Repeated ANOVA method followed by the Post Hoc Bonferroni test.

3. Result and Discussion

Based on the results of the phytochemical test, it can be presented as Table 1 and the results of the in vivo test as Table

Table 1. Phytochemical Test Results

Sample	Phytochemicals			
	Flavonoid (mg/100 gQE)	Tannin (mg/100 g TAE)	Alkaloid	Saponin
P1	1152,597	438,058	++	-
P2	1212,888	772,525	+++	-
P3	1445,784	1150,213	+++	-

Table 2. Results of Lesion Observation

Treatment	Observation result							
	After induction	Score	Day 5	score	Day 10	Score	Day 15	score
K (-)	2 pa	4	1 pu 1 pa	5	2 pu	6	2pu 1 pa	8
	1 pu	3	1 pu 1 pa	5	2 pu	6	3 pu	9
	1 pu	3	2 pu	6	2 pu 1 pa	8	3 pu	9
	2 pa	4	1 pu 1 pa	5	2 pu	6	2 pu 1 pa	8
K (+)	2 pa	4	1 pu	3	1 pa	2	healed	0
	1 pu	3	1 pa	2	healed	0	healed	0
	2 pa	4	1 pu	3	healed	0	healed	0
	2 pa	4	1 pu	3	1 pa	2	healed	0
P0	1 pu	3	1 pa	4	2pa	4	1 pu	3
	2 pa	4	1 pu 1pa	5	2 pa	4	1 pa	2
	3 pu	6	2 pu	6	1 pu 1pa	5	1 pu	3
	2 pa	4	2pa	4	1 pu	3	2 pa	2
P1	1 pu 1pa	5	2 pa	4	1 pu	3	1 pu	3
	1 pu	3	1 pu 1pa	5	1 pu	3	1 pa	2
	1 pu	3	2pa	4	1 pu	3	1 pa	2
	2 pa	4	1 pu 1pa	5	1 pa	2	1 pa	2
P2	1 pu 1pa	5	1 pu	3	healed	0	healed	0
	2 pu	6	2 pa	4	1 pa	2	healed	0
	2 pa	4	1 pa	2	1 pa	2	healed	0
	1 pu 1pa	5	1 pu	3	1 pa	2	healed	0

	1 pu	3	1 pa	2	healed	0	healed	0
P3	1 pu 1pa	5	1 pa	2	healed	0	healed	0
	2 pa	4	1 pu	3	1 pa	2	healed	0
	2 pa	4	1 pa	2	1 pa	2	healed	0

1 = comedo (ko)

2 = papule (pa)

3 = pustules (pu)

Phytochemical test results showed high levels of tannins and flavonoids. The higher the concentration, the higher the levels of flavonoids and tannins. The antioxidant, anti-inflammatory, and photoprotective properties of flavonoids are the main factors influencing their preventive use in the development of skin diseases. Flavonoids accelerate the healing of long-lasting wounds and ulcers due to their circulation-stimulating properties. Plant extracts containing flavonoids are used in creams and solutions to treat and prevent various skin diseases (14). Flavonoids are classified as polyphenols and have anti-inflammatory and anti-acne activities (15). Flavonoids can denature amino acids and enzymes of the P.acne bacteria, thereby damaging the cell wall membrane (16). Flavonoids inhibit bacterial replication because they cause plasma leakage, inhibit bacterial energy metabolism, and cause bacterial cell lysis (17). Flavonoids have antibacterial activity through inhibition of DNA gyrase function so that the ability of bacterial replication is inhibited. This compound will make contact with DNA in the nucleus of bacterial cells. The difference in polarity between the lipids that make up DNA and the alcohol groups in flavonoid compounds causes

damage to the lipid structure of bacterial DNA so that bacteria will lyse and die (18).

Tannins disrupt the PH balance of bacteria by binding to H⁺ ions and inhibiting the reverse transcriptase RNA and DNA topoisomerase enzymes, thereby inhibiting the bacterial replication process and resulting in the inhibition of bacterial growth (16). The mechanism of action of alkaloid compounds as antibacterials is by interfering with the constituent components of peptidoglycan in bacterial cells so that the cell wall layer is not formed intact and causes cell death. In addition, alkaloid components are known as DNA intercalators and inhibit bacterial cell topoisomerase enzymes (19). The mechanism of action of flavonoids as antimicrobials can be divided into 3, namely inhibiting nucleic acid synthesis, inhibiting cell membrane function and inhibiting energy metabolism (20).

Based on the results of statistical tests with Repeated ANOVA and continued with the Post Hoc Bonferroni test, it shows that the Sphericity Assumed sig value <0.05 so that Ha is accepted. This means that there are differences between treatments and administration of supplemented VCO provides healing from time to time

application. The results of the interim study showed that VCO supplementation with intaran extract was able to treat wounds or lesions that appeared due to the induction of P.Acne bacteria. There are differences in each treatment in healing the lesions. Based on the Bonferroni Post Hoc test, there were differences in healing results, namely (1) the negative control had a significant difference with the positive control, P0, P1, P2, and P3; (2) The positive control was significantly different from the negative control, P0 and P1, while not significantly different from P2 and

P3; (3) P0 is significantly different from K-, K+, P2, and P3, but not significantly different from P1; (4) P1 was significantly different from K-, K+, and P3, but not significantly different from P0 and P2; (5) P2 is significantly different from K-, P0, but not significantly different from K+, P1, and P3; (6) P3 is significantly different from K-, P0, P1, but not significantly different from K+ and P2. The statistical test results are then described in graphical form, where in the diagram the numbers of the same letter grades show no significant difference (Figure 2)

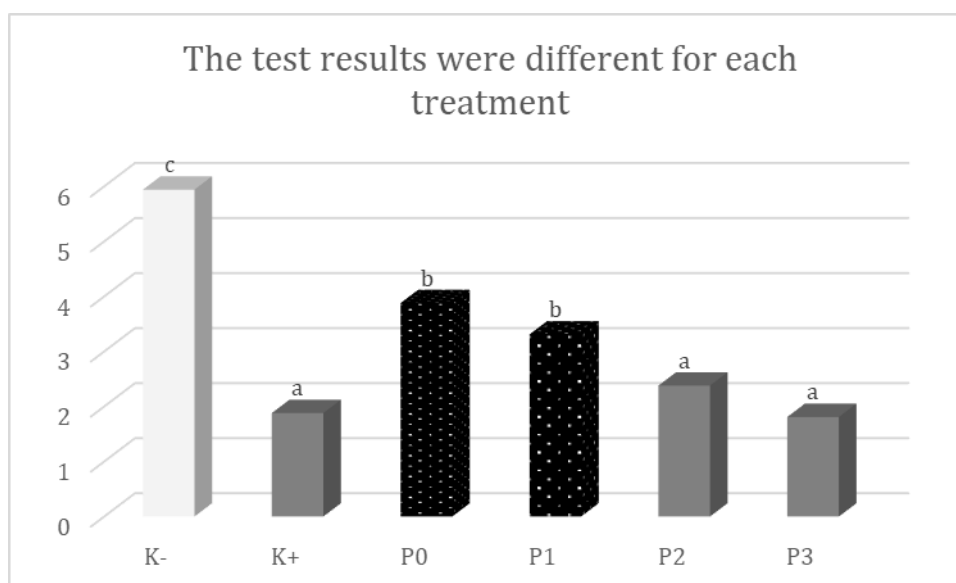


Figure 2. Graph of differences and similarities of each treatment. Different letter codes indicate significant differences (a with b with c), and the same letter codes (a with a, b with b) indicate that there is no significant difference.

The in vivo test analysis was carried out using the Repeated ANOVA method followed by the Post Hoc Bonferroni test. It can be concluded as shown in Figure 2. This graph shows that the lower the graph, the better the speed of healing

of the lesions. Based on the graph, the positive control and P3 showed a high reduction in lesion results compared to other treatments, but not significantly different from P2 or 10% concentration of intaran leaf extract. This

shows that the concentration that can later be used in the formula for making extract emulsions with VCO at 10%. The treatment on VCO and 5% supplementation had a significant difference with the negative control, although it was significantly different from the results on K+. This shows that VCO (P0) can still function as an antibacterial, but not as good as supplemented. VCO in research can act as an antibacterial (21). After the phytochemical test was carried out, it showed that the mixture contained flavonoids, tannins and alkaloids. This formulation is rich in flavonoids making it promising for use in acne treatment and skin protection against premature damage (22).

4. Conclusion

P1, P2, and P3 contained the presence of flavonoids, tannins, and alkaloids, but no saponins. The results showed that VCO-I was able to treat wounds or lesions that appeared due to the induction of P. acne. There are differences in each treatment in healing the lesions. VCO can accelerate the healing of lesions, and it is accelerated by the addition of Intaran extract. Treatment concentrations of 10% and 20% are effective mixtures and have the same healing activity as positive control. To explain the healing due to treatment, it is necessary to do a cytohisto test, so that it can be known tissueally, and it is necessary to continue with clinical trials on humans.

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