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Characterization The Flavonoids Extract of *Tridax Procumbens* l. Leaves and Betel Lime as Materials for Open Wound Analgesic Ointment

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Abstract

Tridax procumbens L. has quite high flavonoid content (6.51%). Flavonoids have antimicrobial effects that can fight bacteria. Besides that, the flavonoid content of *T. procumbens* leaves as an analgesic inhibits has the potential to be used as an analgesic ointment material for open wounds. Betel lime ($CaCO_3$) also helps speed up the healing of open wounds. This research aims to optimize a mixture of *T. procumbens* leaves and betel lime to make a material-based herbal ointment that effective in healing open wounds. This research uses quantitative and qualitative approaches. The dependent variables used are the results of FTIR characterization, SEM-EDX, and antibacterial tests. Research results show that the 1:1 ointment sample has the highest homogeneity with an average particle size distribution of 11.95 nm. The weight and total atomic weight of carbon and calcium elements fluctuated with increasing leaf extract concentration, but the 2:1 ointment sample showed a higher calcium element content than the other samples because the amount of whiting was greater than the leaf extract. Flavonoid functional groups were successfully detected, O-H, C-H, and C-O-C. In *S. aureus*, it was shown that the 1:1 ointment sample was able to inhibit bacterial growth with an inhibition zone diameter of 0.062 to 1.510 cm. In addition, the contents of stigmasterol, β-sitosterol, and n-hexadecanoid acid are the main components that play a role in the inhibitory activity of bacteria.

Keywords: Analgesic ointment, flavonoids, open wounds

1 Introduction

Open wounds are damaged structures and anatomical functions of the skin, so proper treatment is required to avoid infection due to contamination by germs or bacteria.

There are various types of open wounds, such as punching, lasering, cracking, injection, abrasion, and excision wounds [1]. Excision and incision wounds are caused by sharp scratches, excision wounds accompanied by epidermal tissue cuts while incision injury is not [2]. In 2013, the number of incision injuries reached 23.2 per cent and in 2018 it increased to 25.4 percent [3]–[5].

Open wound healing consists of phases of hemostasis, inflammation, proliferation, and remodelling. This phase can occur naturally, but if the treatment and maintenance is not optimal it will cause wider tissue damage [6]. Most of the treatments for wound healing are with the administration of ointment. There are many kinds of special wound ointments, this variation depends on the composition of the ingredients and the price. Regularly marketed ointments often contain the chemical povidon iodine that has irritating side effects on the skin [7]. On the other hand, continuous use of antibiotic ointment can cause resistance if its use is not based on proper instructions [8].

Indonesia has a variety of plants that can be used in the treatment of open wounds, one of which is the leaves of *T. procumbens* L. [9]. The contents of these plant compounds include the alkaloids aquamidine, voacangine, flavonoids procumbentin, luteolin, quercetin, isokuersetin, phenol, and saponins [10]. Based on the contents, the plant *T. procumbens* is potentially used as antimicrobial and antioxidant, anti-inflammatory, anticancer, antidiabetic, antihypertensive, immunomodulator, and hepatoprotector [10], [11]. Based on research by [12] it also shows that the plant has antimicrobial effect that is capable of fighting gram-positive and gram-negative bacteria so that it can heal wounds. Therefore, based on the properties and content of *T. procumbens* leaves potentially used as one of the materials of open wound analgesic ointment. However, the research has a weakness, namely that there are no substances that help wounds dry quickly, such as turnip. Turnip consists of calcium carbonate ($CaCO_3$) that helps accelerate open wound healing [13]. Based on a study by [14] that combines turnips and turnipple extract with a ratio of 2:1 more rapidly heals wounds and inhibits inflammation than 1:1 and 1:2. The research aims to optimize the mixture of *T. procumbens* leaves and turnip for making nanomaterial-based herbal ointments so that they can be effective in healing open wounds.

2 Material and Methods

The Place and Time

Research was carried out in July until November at the Laboratory of Material Physics, Integrated Laboratory for Biology of Faculty Mathematics and Natural Sciences, the Central Laboratory on Minerals and Advanced Materials of the Universitas Negeri Malang.

Equipment and Materials

The materials used in this research include *T. procumbens* leaves, betel lime, ethanol, 70% alcohol, aquades, water, DI water, CMC (carboxy methyl cellulose), vaseline flavum, adeps lanae, propylene glycol, Methyl paraben, paraben propyl, BHT (butylated hydroxytoluene), spirtus, lisol, and medium Na. For the equipment used include glass, filter paper, pH paper indicator, alumunium foil, handcuffs, analytical weights, ovens, hot plates, magnetic stirrer, blenders, pipettes, stopwatches, glass glasses, erlenmeyer, paper disks, and OSE needles.

Research Variable

The free variable used is the dose of *T. procumbens* leaf extract. The controlled variables used are the dosage of calcium, the amount of vaseline flavum and adeps lanae, the duration of drying, and the rate of decomposition. The bound variables used are the results of the characterization tests FTIR, SEM-EDX, and antibacterial tests.

This research uses a combination of quantitative methods by conducting experiments in the laboratory and qualitative with library studies. The technique is intended to make an analgesic ointment medication from extracts of *T. procumbens* leaves and betel lime.

Characterization Techniques and Data Analysis

The characterization techniques carried out are FTIR, SEM-EDX characterization, antibacterial testing, and effectiveness (analgesic strength) and antibacteria strength. Data analysis to determine the effect of the comparison of extracts with betel lime using Oneway ANOVA with further testing using the Duncan test with a degree of significance of 5%. Data analyzed using SPSS 25.

3 Results and Discussions

The Material of The Analgesic Ointment

In this research consists of extracts of *T. procumbens* leaves, betel lime, CMC, DI water, BHT, vaseline flavum, adeps lanae, methyl paraben, propyl parabens, and propylene glycol. *T. procumbens* leaf extract acts as an active substance in the healing of open wounds because it contains flavonoids that are analgesic [15]. It also acts as an active ingredient because it contains calcium carbonate ($CaCO_3$) which can also help speed up the healing of open wounds [13]. This analgesic ointment is made in three variations with a comparison of the composition of *T. procumbens* leaf extract and betel lime 1:2, 1:1, and 2:1.

CMC serves as a gel base for ointments and DI water as an extract solvent. Meanwhile, BHT acts as an antioxidant to prevent damage to the ointment mixture due to oxidation [16]. Further, there is a vaseline flavum acting as a base of hydrocarbon ointment which has the advantage of being able to last on the skin for a long time, while adeps lanae as a basis of water-absorbing ointments has good softening properties (emolien) on skin [17]. Paraben and methyl parabens function as preservatives, making the ointment last longer and have good antibacterial properties. Propylene glycol itself acts as a humectant which means it can repair and maintain the stability of the ointment over a long period of time [16]. Overall, the final formulation of ointments can be seen in Table 1 below.

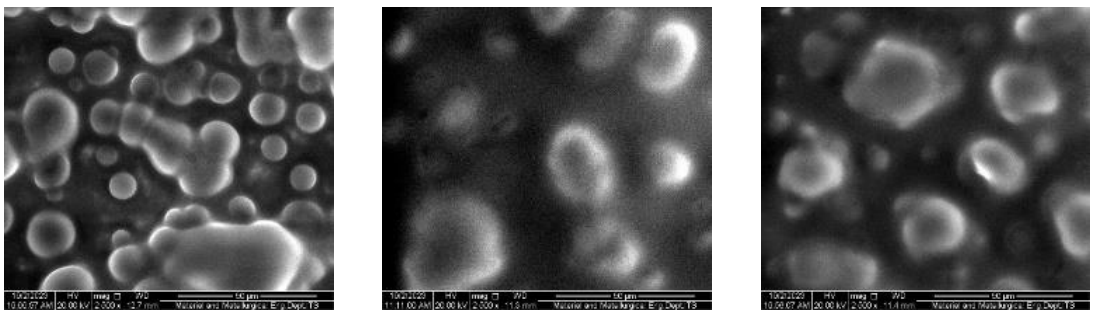
Table 1. Final Formulation of Ointments

No	Mixed Type	Material	Function	Variation		
				1:2	**1:1**	**2:1**
1	Type A	Leaves extract (g)	Active substance	12	12	12
		CMC (g)	Gel base	1	1	1
		DI water (mL)	Solvent	100	100	100
Total (g)				-	-	-
2	Type B	Type A (g)	Active substance	2,5	5	10
		Betel lime (g)		5	5	5
Total (g)				7,5	7,5	7,5

3	Type C	Campuran B (g)	Active substance	2,67	2,67	2,67
		BHT (g)	Antioxidant	0,1	0,1	0,1
4	Type D	Adeps lanae (g) Vaseline flavum (g)	Ointment base	8	8	8
		Propil paraben (g)	Preservative	0,05	0,05	0,05
5	Type E	Metil paraben (g)		0,18	0,18	0,18
		Propilen glikol (g)	Humectant	1	1	1
Total (g)				**12**	**12**	**12**

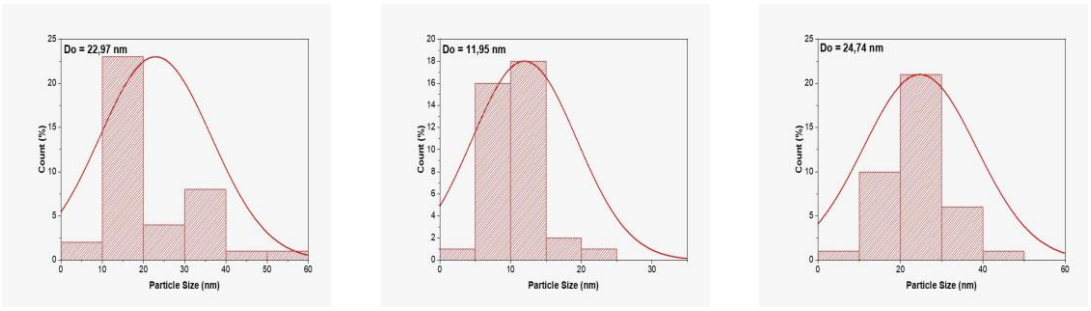
Morphology, Size, Distribution, and Content of Analgesic Ointment Elements

The surface area and bioactivity of the synthetic material are largely influenced by its size and shape. The results of SEM characterization of analgesic ointments 1:2, 1:1, and 2:1 show that the surface morphology is dominantly round, longitudinal, and has no crystalline structure as shown in Figs. 1 (a)-(c). Figs 2 (a)-(c) shows the distribution of the nanoparticles of the analgesical ointment has varying sizes. Ointments with a composition of 1:1 have high homogeneity with a particle size distribution of 11.95 nm, smaller than 1:2 and 2:1 with values of 22.97 nm and 24.74 nm. This is due to molecular clotting on 1:2 and 2:1 due to too thick polishing on the prepared glass. The effectiveness of analgesic ointment compared to reverse particle size. The smaller the particle size, the higher the surface area and the better its penetration so it's easier to penetrate the layer of the epidermis as well as interact with the wound cells and increase the rate of drug release. A homogeneous ointment is characterized by the absence of clamps on the treatment result, a smooth structure and a uniform colour from the starting point to the end point of the treatment [18].



(a) (b) (c)

Figure 1. SEM Characterization Results of Analgesic Ointment



(a) (b) (c)

Figure 2. Results of Analysis of Distribution and Particle Size of Analgesic Ointment

EDX analysis with mapping is used to identify elements in analgesic ointments 1:2, 1:1, and 2:1. The presence of the elements that make up the active ingredients of the ointment such as carbon, oxygen, and calcium in all the pure ointments samples has been identified. There is a predicted contaminant such as silicone (Si) originating from the glass preparation used in the SEM-EDX characterization. The total atomic weight of the carbon and calcium elements increased as the concentration of the leaf extracts increased, but in 1:2 the calcium element content was higher than in the other samples because the number of betel lime was more than in leaf extract. Table 2 shows the different weight and atomic proportions of the elements 1:2, 1:1, and 2:1 synthesized. Although there are differences in the element content of each sample of the ointment, the differences are not very significant due to the substance content of almost the same active substance. The research also concluded that the process of combining and making the ointment produces a high and effective purity.

Table 2. Chemical Element Composition of Analgesic Ointment

Element	**1:2**		**1:1**		**2:1**	
	Wt%	***At%***	***Wt%***	***At%***	***Wt%***	***At%***
C	83,89	89,32	86,98	90,68	84,35	89,48
O	11,19	8,94	10,84	8,48	10,72	8,54
Si	1,27	0,58	1,18	0,53	3,06	1,39
Ca	3,65	1,16	1,00	0,31	1,87	0,59
Total (%)	**100**	**100**	**100**	**100**	**100**	**100**

FTIR Spectrum Analgesic Ointment Function Group of analgesic ointment is shown by Fig. 3. The extension of the O-H function cluster of the phenol function was detected at the wave number 3644-3233 cm–1. C=O aryl ketone stretching was detected at 1684-1682 cm–1. The C=C aromatic ring stretching tape was visible at 1615-1520 cm–1. The C-H sliding tape in aromatic hydrocarbons was detected at 2965-2859 cm– 1 and 1393 cm– 1. Tires at 1180, 1163, and 1285-1002 cm-1 were caused by the C–O stretching of the aryl ether ring, the C-O stretch of the phenol, and the C = O-C stretching in each of the ketones. The C–C stretch tape was successfully detected on 1652-1650 cm– 1, whereas the =C-O-H tape was detected in 1385-1336. Tires of the C-O-C were also detected in 1285-1163 and 831-824 cm-11. These functional groups are a functional group of flavonoids such as quercetin and kaempferol that act as analgesic materials. The function group values are also fairly consistent as by [19], [20] (Tabel 3).

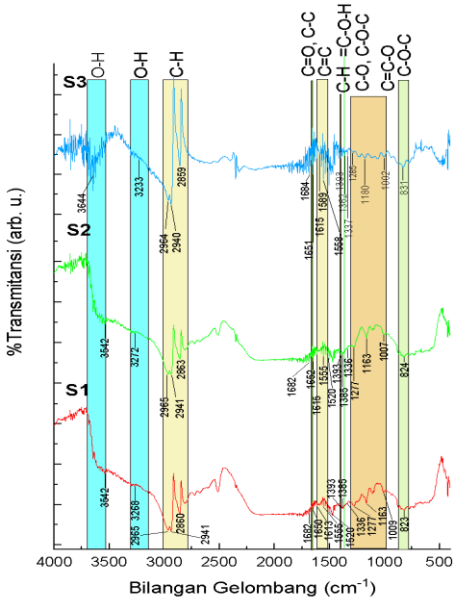


Figure 3. FTIR Test Results of Analgesic Ointment.

Tabel 3. Results of FTIR Analysis of Analgesic Ointment.

QUERCETIN				
Functional Groups	**Wave Number (cm^{-1})**			**Reference**
	1:2	**1:1**	**2:1**	
O-H	3542 – 3268	3542 – 3272	3644 – 3233	[19]
C-H	2965, 2941, 2860	2965, 2941, 2863	2964, 2940, 2859	
C=O	1682	1682	1684	

C=C	1613, 1555, 1520	1615, 1555, 1520	1615, 1589, 1558	
=C-O-H	1385, 1336	1385, 1336	1362, 1337	
C-O-C	1277, 1163, 824	1277, 1163, 824	1285, 1180, 831	
C=C-O	1277, 1007	1277, 1009	1285, 1002	
KAEMPFEROL				
Functional Groups	**Wave Number (cm^{-1})**			**Reference**
	1:2	**1:1**	**2:1**	
O-H	3542 – 3268	3542 – 3272	3644 – 3233	[20]
C=O	1682	1682	1684	
C-C	1650	1652	1651	
C-H	1393	1393	1393	
C-O	1163	1163	1180	

Table 4. Antibacterial Inhibitory Power ff Analgesic Ointments

Bacteria	**Inhibition Zone Diameter (cm) ± St. Dev**		
	1:2	**1:1**	**2:1**
Escherichia coli	1,033 ± 0,005[a]	1,503 ± 0,015[b]	1,046 ± 0,232[a]
Staphylococcus aureus	0,386 ± 0,557[a]	1,406 ± 0,020[b]	1,073 ± 0,015[b]

Description: A number followed by the same letter in the same column, no real difference based on the Duncan test with a 5% significance rate (average ± stdev).

Bacterial Resistance

Here is the result of the analysis of the bacterial resistance of the base of the ointment with the main ingredient of the extract of the *T. procumbens* leaf with the purple betel lime (Table 4).

On a sample of *S. aureus* bacteria, a preparation of ointment with an extract ratio of 1:1 between *T. procumbens* leaves and lime can inhibit the growth of bacteria with a barrier zone diameter of 0.062-1,510 cm. In addition, there is a barrier zone that has been formed but has been rebuilt by bacteria. This can be caused by the concentration of bacteria and the number of the bacteria [21]. *E. coli* is one of the normal organisms that can cause infection [22]. This bacterium has a barrier zone of 1,029-1,520 cm. Comparing

the preparation of the ointment to a 1:1 ratio indicates a wider barrier zone compared to a 1:2 and a 2:1. It is believed to result from the release of Ca^{2+} and K^{+} ions from inside the bacterial cell membranes, resulting in cell leakage and changes in the morphology of *E. coli* cells. In addition, stigmasterol, β-sitosterol, and n-hexadecanoid acid are major components that play a role in the barrier activity of gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria [9]. The compound spreads the spectrum of antibacterial activity on bacteria [23].

4 Conclusions

The ointment from the extracts of *T. procumbens* leaves and betel lime has the characteristics of surface morphology that are dominant round, longish, and have no crystalline structure and have high purity properties. The distribution and size of the ointment also varies where 1:1 samples have the highest homogeneity with a particle size distribution of 11.95 nm. Flavonoid function groups (curcetin and kaempferol) have been successfully identified, indicating that the ointment is analgesic so it is effective for healing open wounds. The ointment has fairly good antibacterial properties, where 1:1 samples have the best antibacteric properties with an average barrier zone diameter of 1,406 ± 0,020b for gram-positive bacteria (*S. aureus*) and 1,503 ± 0.015b for Gram-negative bacteria (*E. coli*).

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