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Formulation of ointment from extract and fractions of *Castanopsis costata* leaves

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Abstract

Background: *Castanopsis costata* (Blume) A. DC. or commonly known as cep-cepan is a medicinal plant widely used based on experience by Karo people in Indonesia n the Karo community for the treatment of wounds. However, no research has been done to prove this experience. Initially, the formulation of ointment extract and leaf fractions of *C. costata* need to be carried out.

Aim: The purpose of this study was to provide information about the chemical group content contained in *C. costata* leaf extract and fractions and to evaluate their ointment preparation according to the requirements of ointment preparations.

Method: The extraction of *C. costata* leaves using 70% ethanol using the maceration method followed by the fractionation of *C. costata* leaves using water, ethyl acetate, and n-hexane as solvents and phytochemical screening were carried out. Furthermore, the preparation of ointments and evaluation of extract ointment preparations and *C. costata* leaf fraction ointment was performed.

Result: The results indicated that the ethanol extract of *C. costata* contained alkaloids, flavonoids, glycosides, steroids/terpenoids, saponins and tannins, while the water fraction contained flavonoids, saponins and tannins. The ethyl acetate fraction contained flavonoids, steroids/terpenoids, saponins, and tannins and the n-hexane fraction contained alkaloids and steroids/terpenoids. The results also indicated that the extract and fraction ointment preparations and ointment fractions evaluation, including organoleptic, homogeneity, and pH, met the evaluation requirements for ointment.

Conclusion: Ethanol extract of *C. costata* contains alkaloids, flavonoids, glycosides, steroids/terpenoids, saponins and tannins, while the water fraction contains flavonoids, saponins and tannins. The ethyl acetate fraction contains flavonoids, steroids/terpenoids, saponins, and tannins and the n-hexane fraction contains alkaloids and steroids/terpenoids. The extract and fraction ointment preparations and ointment met the evaluation requirements for ointment.

Keywords: *Castanopsis costata*; Phytochemical screening; Extract; Fractions; Ointment

1. Introduction

Indonesia has the largest biodiversity in the world, with 30,000 plant species with medicinal properties. One of them is the species *Castanopsis costata* or commonly known as cep-cepan which is widely used by the Karo community and is still used based on experiences such as for the treatment of wound, fever and stomach ache [1]. The development of

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pharmaceutical preparation for this plant is needed to increase its use [2]. Ointment is one of the pharmaceutical preparations that are easy and practical to use. Ointment is a preparation in which drug substances are dispersed in base as a carrier. The most commonly used ointment bases are vaseline album and adeps lanae [3].

2. Material and Methods

2.1. Materials

The materials used in this study were *Castanopsis costata* (Blume) A. DC. leaves, 70% ethanol, ethyl acetate, n-hexane, aquadest, ammonia, chloroform, hydrochloric acid, Mayer's reagent, Dragendorff's reagent, magnesium powder, amyl alcohol, reagent iron(III) chloride, methanol absolute, ether, acetic acid anhydrous, concentrated sulfuric acid, benzene, sodium chloride, lead(II) acetate, isopropanol, Molish reagent, Liebermen-Bouchard reagent.

2.2. Sample Preparation

The leaves of *C. costata* are cleaned with running water, air-dried and protected from direct sunlight. Then the *C. costata* leaves were mashed using a blender and placed in a plastic container to limit the effects of moisture and other contamination, and then stored at room temperature.

2.3. Determination of Water Content

Water content was determined by the azeotropy method (toluene distillation). 200 ml of toluene and 2 ml of distilled water were put into a round bottom flask, distilled for 2 hours, then cooled for 30 minutes, and the volume of water in the receiving tube was read to the nearest 0.05 ml. Dried-sample was weighed as much as 5 g and put into a round bottom flask containing saturated toluene, heated for 15 minutes, after the toluene boiled, the distillation was adjusted to 2 drops per second until all the water was distilled. Distillation was continued for 5 minutes, then the receiving tube was allowed to cool to room temperature. After the water and toluene have completely separated, the volume of water is read according to the water content contained in the dried sample.

2.4. Preparation of Extract and Fractions

As much as 2000 g of *C. costata* leaf simplicia powder was extracted using the maceration method using 70% ethanol for five days (5x24 hours). Then the macerate was collected, and the solvent was evaporated using a rotary evaporator at 50^{o}C to become thick. The ethanol extract of *C. costata* leaves was fractionated using different solvents, namely non-polar solvents such as n-hexane, semi-polar such as ethyl acetate and polar solvents such as water. The extract was weighed and dissolved in n-hexane solvent in a separatory funnel and then left for a while until separation occurred to obtain a clear layer of n-hexane. Then, the n-hexane fraction was collected, and the resulting residue was added to ethyl acetate and was shaken and left for a while until separation occurred. The ethyl acetate layer was collected and the resulting residue was used for the fractionation with water, the residue was shaken and left for a while to obtain a clear water layer. Then each fraction was evaporated with a rotary evaporator to obtain a viscous fraction.

2.5. Preparation of Ointment Extract and Fractions

The ointment base used in this study were adeps lanae and vaseline album [3]. Table 1 shows the ointment formulas of the extract and fractions.

Table 1 Ointment formulation of extract and fractions of *C. costata* leaves**.**

Composition	**15%**	**30%**	**45%**
Extract/Fraction	7.5	15	22.5
Ointment basis	42.5	35	27.5
Total	50 g	50 g	50 g

2.6. Evaluation of Ointment Preparations

2.6.1. Organoleptic test

Organoleptic testing was performed by visually observe at the preparation from texture and color [4].

2.6.2. Homogeneity test

As much as 0.5 g of ointment was placed on a glass object then it was flattened and observed visually. The homogeneous ointment was characterized by no lumps at the time of application [5].

2.6.3. pH test

The pH of the ointment preparation was measured by dipping the pH meter into the diluted ointment preparations. The pH value was seen on the scale in the tool and recorded after the stable pH value was observed [5]. A good ointment pH is 4.5-6.5 according to the pH value of human skin [6].

3. Results and Discussion

3.1. Determination of Water Content

The water content of *C. costata* leaf simplicia was 8.56%, which fulfilled the requirements for simplicia water content which is less than 10% [7].

3.2. Extract and Fractions

The ethanol extract obtained from 2000 g of dried sample was 345 g (17.25% w/w) which met the standard of the Ministry of Health of Indonesia (7.2%) [9]. The ethanol extract of *C. costata* leaves was fractionated liquid-liquid using solvents with different polarities. The water fraction obtained from 250 g of ethanol extract was 62 g (24.8% w/w). The ethyl acetate fraction obtained from 287 g of ethanol extract was 62 g (21.6% w/w), and the n-hexane fraction obtained from 200 g of ethanol extract was 53 g (26.5%).

3.3. Phytochemical Screening of Extract and Fractions of *C. costata* Leaves

The results of the phytochemical screening test of *C. costata* leaf extract and fractions can be seen in Table 2.

Table 2 Phytochemical screening of extract and fractions

Secondary Metabolite	Ethanol Extract	Water Fraction	Ethyl acetate Fraction	n-hexane Fraction
Alkaloids	+	-	-	+
Flavonoids	+	+	+	-
Glycosides	+	-	-	-
Steroids/Terpenoids	+	-	+	+
Saponins	+	+	+	-
Tannins	+	+	+	-

Information: +: Contains the metabolite-: Does not contains the metabolite

3.4. Evaluation of Extract and Fractions Ointment Preparations

3.4.1. Organoleptic test

Observation of organoleptic of ointment preparations of extract and fractions of *C. costata* leaf consisted of shape, odor and color. The organoleptic test results are listed in table 3.

Table 3 Organoleptic Test Results

Formulation	Form	Color	Smell
Extract 15%	Half solid	Dark brown	Typical smell of Cep-cepan
Extract 30%	Half solid	Dark brown	Typical smell of Cep-cepan
Extract 45%	Half solid	Dark brown	Typical smell of Cep-cepan
Water fraction 15%	Half solid	Dark brown	Typical smell of Cep-cepan
Water fraction 30%	Half solid	Dark brown	Typical smell of Cep-cepan
Water fraction 45%	Half solid	Dark brown	Typical smell of Cep-cepan
Ethyl Acetate fraction 15%	Half solid	Brownish green	Typical smell of Cep-cepan
Ethyl Acetate fraction 30%	Half solid	Brownish green	Typical smell of Cep-cepan
Ethyl Acetate fraction 45%	Half solid	Brownish green	Typical smell of Cep-cepan
n-hexane fraction 15%	Half solid	Light green	Typical smell of Cep-cepan
n-hexane fraction 30%	Half solid	Light green	Typical smell of Cep-cepan
n-hexane fraction 45%	Half solid	Light green	Typical smell of Cep-cepan

The form of the extract ointment and the *C. costata* leaf fraction ointments were in accordance with the criteria for the form of the ointment, namely semi-solid.

3.4.2. Homogeneity Test

The homogeneity of the extract and fractions ointment showed that the preparations were homogeneous. The homogeneity test results can be seen in table 4.

Table 4 Homogeneity Test Results

Formulation	Homogeneity
Extract 15%	Homogeneous
Extract 30%	Homogeneous
Extract 45%	Homogeneous
Water fraction 15%	Homogeneous
Water fraction 30%	Homogeneous
Water fraction 45%	Homogeneous
Ethyl Acetate fraction 15%	Homogeneous
Ethyl Acetate fraction 30%	Homogeneous
Ethyl Acetate fraction 45%	Homogeneous
n-hexane fraction 15%	Homogeneous
n-hexane fraction 30%	Homogeneous
n-hexane fraction 45%	Homogeneous

The homogeneity of the ointment was indicated by the absence of agglomerates, which indicated that the mixing of the basic ingredients of the ointment and the extract/fraction of *C. costata* leaves was quite good so that there were no lumps [8]. Homogeneous ointment preparations will give good results because the medicinal ingredients are dispersed

in the basic ingredients evenly, so that each part of the preparation contains the same amount of medicinal ingredients [9].

3.4.3. pH test

The pH results of the extract and fractions ointment can be seen in table 5.

Table 5 pH test results

Formulation	pH
Extract 15%	5.6
Extract 30%	5.6
Extract 45%	5.6
Water fraction 15%	5.5
Water fraction 30%	5.6
Water fraction 45%	5.6
Ethyl Acetate fraction 15%	5.7
Ethyl Acetate fraction 30%	5.5
Ethyl Acetate fraction 45%	5.7
n-hexane fraction 15%	5.4
n-hexane fraction 30%	5.4
n-hexane fraction 45%	5.4

Based on results, the pH of the ointment preparations of the extract and fractions were 5.4-5.7, which met the pH requirements between 4.5-6.5. Topical preparations should have the same pH as normal skin. The suitability of the skin's pH with the preparation affects the skin's acceptance of the preparation. The ideal topical preparation is not irritating to the skin, but skin irritation might be possible if the preparation is too acidic or too alkaline [9].

4. Conclusion

In conclusion, the ethanol extract of *C. costata* leaves contains alkaloids, flavonoids, glycosides, steroids/terpenoids, saponins and tannins. The water fraction of *C. costata* leaves contains alkaloids, saponins and tannins, while the ethyl acetate fraction contains flavonoids, steroids/terpenoids, saponins and tannins. Meanwhile, the n-hexane fraction of *C. costata* leaves contains alkaloids and steroid/terpenoid compounds. The ointment preparations of extract and fractions of *C. costata* meet the requirements.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

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