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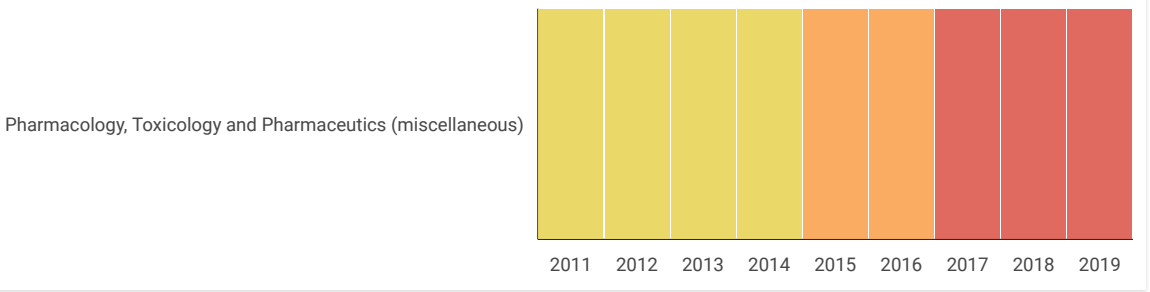

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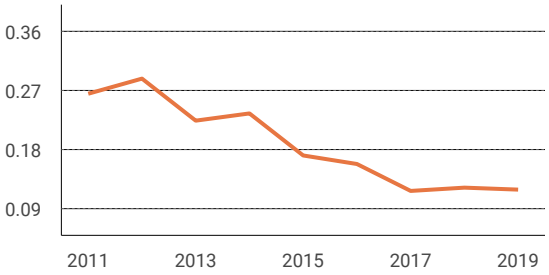

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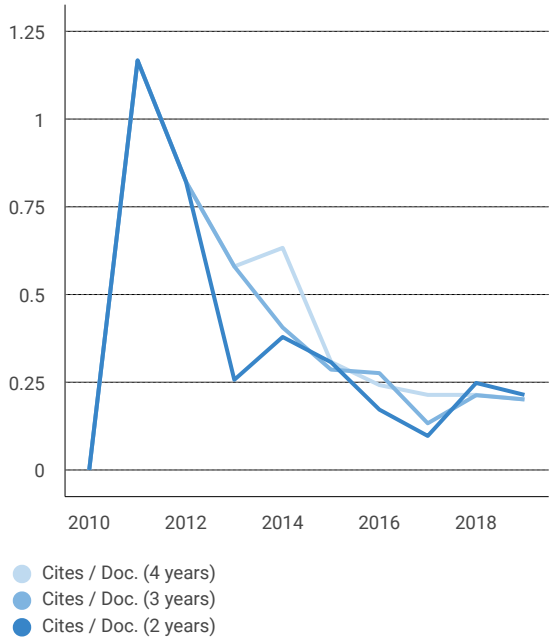

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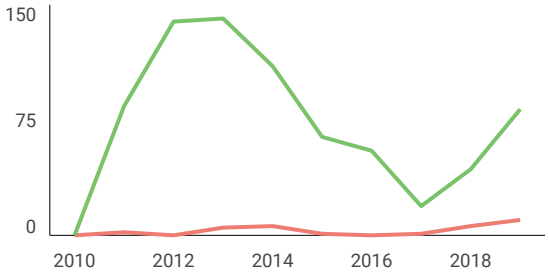

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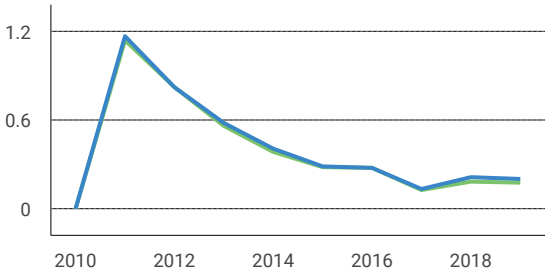

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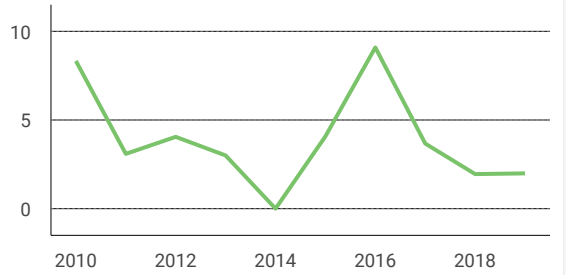

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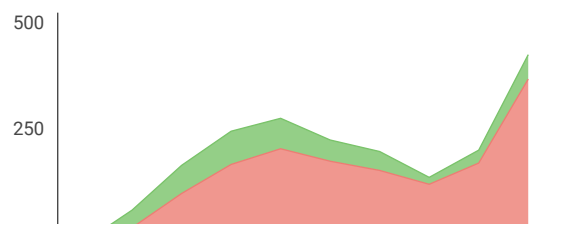

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Characterization and antibacterial activity of cocos Nucifera L. Meat extract and powder as a drug and cosmetic agent

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ABSTRACT

Cocos nucifera L. or Kopyor coconut is the natural material plant that has nutrient content, including carbohydrates, proteins, fats and fatty acids. The potential of Kopyor coconut is mainly produced from water and soft flesh or meat. Some benefits of the meat have not been widely studied as an active drug agent. This study was aimed to identify and characterize kopyor meat extract and to test activity as an antibacterial agent. The maceration method was used to extract kopyor meat. The optimized extraction resulted in a yield of 23% efficiency. Kopyor meat extract was identified in terms of loss on drying, total ash, acid-insoluble ash, extract content and saponification value. Evaluation of the antibacterial activity of both dried meat kopyor and extract were conducted. The standardized extract had a loss on drying of 35%, total ash of 8.95%, acid-insoluble ash of 31%, and extract content soluble in water and ethanol of 56.9% and 0.6% respectively. The saponification value showed a value of 56. It was shown that both powder and Cocos nucifera extract had as same high activity as an antibacterial agent against Staphylococcus aureus, Pseudomonas aeruginosa and Staphylococcus epidermidis therefore this recommended for further formulation and evaluation as drug and cosmetic.

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INTRODUCTION

Cocos nucifera L. or coconut kopyor is a plant that almost all parts of the plant have been used by humans. Therefore, it is considered a versatile plant, especially for coastal communities and is a type of plant from the Arecaceae. The most widely used main component of coconut kopyor is young coconut water and soft flesh. Some of the benefits are kopyor coconut water for antipyretic and anti-inflammatory activities (Mantena *et al.*, 2003; Xiao *et al.*, 2017). While the benefits of the fruit flesh themselves have not been widely studied as medicinal or cosmetic ingredients. The content of the main bigger components in the fruit flesh is hypothesized to produce pharmacological activity as a medicinal and cosmetic ingredient anti-oxidants, anti-osteoporosis, antidiabetic, antineoplastic, bactericidal, antihelminthic, antimalarial, leishmanicidal, antifungal, and antiviral activities (Ajeet *et al.*,

2017; Lima *et al.*, 2015; Alleyne *et al.*, 2005). To be able to provide great benefits in developing its potential as a medicinal and cosmetic ingredient, Kopyor coconut extract needs to be extracted with the proper method, which needs to be identified and characterized by all the main components contained. The utilization was mostly still in water or oil. Extracts of Kopyor coconut were hypothesized and indicated for anti-itching, anti-bacterial, antioxidant, anti-viral, analgesic and anti-inflammatory drugs (Lima *et al.*, 2015). While the benefits in cosmetics for skin and hair care can also reduce psoriasis, eczema, soften the skin, reduce skin dryness, prevent wrinkles and black spots as well as anti UV radiation (Esquenazi *et al.*, 2002). The extraction process is the first process before kopyor being tested for pre-formulation, formulation and further activity testing. Extraction is a process of extracting an active compound from material or simplicia using a suitable solvent. Extraction can be done by various methods, according to nature and purpose. This research will study the identification, characterization and antibacterial activity of kopyor coconut extract (Cocos nucifera L.).

MATERIALS AND METHODS

Cocos nucifera L. extract and dried kopyor, aquadest, methanol (p.a), hexane (p.a), HCl (p.a), NaOH (p.a), $FeCl_3$ (p.a), filter paper (Whatman No. 1).

Sample Preparation

Samples of kopyor powder (Cocos nucifera Linn) were obtained from the Coconut Research Center in Purwokerto. Samples of this research were divided into kopyor powder and extract.

Extraction of Cocos nucifera L. by maceration method using 70% ethanol

Kopyor powder samples (Cocos nucifera L) were weighed as much as 300 grams. Samples were put in a maceration container and 70% ethanol containing 1% HCl with a ratio of 1: 4 (w/v) was added until all submerged and tightly closed, followed by keeping for 24 hours and stirring several times during maceration. Samples were filtered using filter paper Whatman No. 1 and were separated. The filtrate was then macerated again with a new solution of 70% ethanol containing 1% HCl. This process was done three times with each time of 24 hours. The extract obtained was evaporated at 50°C and a speed of 80 rpm until a thick extract was obtained.

Phytochemical Screening Analysis

Alkaloid Test

The Alkaloid Test was carried out by the Dragendorff method. A sample of 3 ml was placed in a test tube and then 5 ml of methanol was added, followed by the addition of 3 ml of ammonia to a pH value of 8-9. The methanol extract was filtered and 2 ml of sulfuric acid was added and was shaken to get 2 layers. The top layer (sulfate) was taken 5 drops, then 1 drop of Dragendorf reagent was added and the formation of orange deposits showed the presence of alkaloids (Rao and Mohd, 2016).

Saponin Test

The Saponin test was carried out using the Forth method by inserting 2 ml of the sample into a test tube and then 10 ml of distilled water was added. The mixture was heated for 2-3 minutes and was cooled down. After being shaken for 30 seconds, changes occurred. If the permanent solid foam was formed (permanently for 30 seconds), this indicated the presence of saponins (Rao and Mohd, 2016).

Flavonoid Test

The flavonoid test was carried out using the Shinoda method. A total of 0.5 ml of the sample was dropped on a glass preparation. Next, 3 drops of methanol were added and were stirred until homogeneous. Following that, a small piece of Mg tape was added, then 3 drops of concentrated HCl were added. The formation of yellow, orange, red, or blue indicated the presence of flavonoid compounds.

Phenolic Test

A total of 0.50 ml of sample was dropped on a glass preparation, then 3 drops of methanol were added and were stirred until homogeneous, followed by the addition of 3 drops of 5% $FeCl_3$. The formation of green, red, purple, or blue indicated the presence of phenolic compounds (Rao and Mohd, 2016).

Flavonoid Test using Thin Layer Chromatography (TLC)

The filtrate on phytochemical screening was plated on silica gel 60 F254 plates, then it was rubbed with a mixture of butanol: acetic acid: water at ratio 3: 1: 1, then it was dried and was observed using 254 nm and 366 nm UV light. Furthermore, the plates were sprayed with ammonia, were dried and were re-observed with 254 nm and 366 nm UV light (Rao and Mohd, 2016).

Anthocyanine Content Test

This method was used to test the existence of anthocyanin. The first method was by heating with 2M HCl for 2 minutes at 100°C, then the sample color was observed. If the red color in the sample did not change (steady), this showed the presence of anthocyanin. The second way was by adding samples with drops of 2M NaOH. If the red color turns

blue-green and fades slowly, it was indicated anthocyanin (Anggriani *et al.*, 2017).

Characterization of Kopyor Extract

Moisture Content

The moisture content of the extract was measured using the Moisture Content Analyzer after the drying process with Freeze Dryer.

Extract standardization and characterization

Determination of Ash Levels

2 grams of the refined test material was weighed, and then it was inserted into the silicate crucible, which has been glowed and anchored. Spread it slowly until the charcoal runs out in the furnish temperature of 800°C. The crucible was then cooled and weighed. If the charcoal cannot be removed, enough hot water was added. Hot water was stirred and then filtered using ash-free filter paper. The filtrate was refined along with filter paper in the same silicate crucible in the 800°C and it was furnished to a fixed weight. The total ash content was measured and was expressed in % w/w.

Determination of Non-Soluble Acid Levels

The ash obtained in the determination of total ash content was boiled with 25.0 mL dilute HCl for 5 minutes. Insoluble parts were gathered in acid using ash-free filter paper. The filter paper was rinsed with enough hot water and spread the ash-free filter paper into the crucible in the furnish temperature of 800°C to a fixed weight. The insoluble ash content in the acid was calculated against the weight of the tested samples and was expressed in % w/w.

Determination of Water-Soluble Extract Content

5 grams of dried powder was weighed and inserted into Erlenmeyer. 100.0 mL of chloroform saturated water (water: chloroform = 1: 1) was added and was shaken many times for 6 hours. This mixture was then left for 18 hours and was filtered. The 20.0 mL of filtrate was filtered in a dish that has been anchored at 105°C. Heating the filtrate into the oven at 105°C until the weight remains and the level of the water-soluble extract was calculated in %w/w.

Determination of Ethanol Soluble Extract Content

5 grams of dried powder was weighed and inserted into Erlenmeyer. 100.0 mL of concentrated ethanol pro analysis was added and was shaken many times for 6 hours. This mixture was then left for 18 hours and was filtered. The 20.0 mL of filtrate was filtered in a dish that has been anchored at 105°C. Heating the filtrate into the oven at 105°C until the weight remains and the level of the ethanol-soluble extract was calculated in %w/w.

Determination of loss on drying

1 gram of extract was weighed in a weighing bottle that has been anchored and dried at 105°C. The smooth extract was flattened by tapping the weighing bottle onto the floor. The weighing bottle was inserted into the oven at 105 °C. Weight up to constant weights and was expressed in % w/w

Saponification value

2.5 grams of sample in Erlenmeyer 250 mL was weighed. 25.0 mL of KOH - ethanol 0.5 NLV was added. The mixture was continued to reflux for 30 minutes. Then 1 mL of the PP indicator was added. The excess of KOH was titrated using 0.5 N HCl. The saponification value was calculated.

Characterization of fatty acid content

The identification of fatty acid content in kopyor extract by the GC-MS method was conducted. Methyl esters from fatty acids were made by dissolving samples in HCl (1.5 M, 15 mL) in methanol. The sample solution was then refluxed at 60ºC for 2 hours using a water bath. Then toluene (1 mL) was added, the solvent was then evaporated in the rotary evaporator. The FAME reaction was purified with SiO2 eluted with hexane / EtOAc (1/1) to obtain FAME derivatives, which were then analyzed by GC-MS.

The GC-MS analysis was performed on GC-FID with Agilent Technologies 6890N and GC-MSD with the Agilent 6973 series equipped with the Willey 7n.1 database in the HP-5 column (30 mx 0.250 mm x 0.25 μm). The temperature for GC-MS starts at 100ºC, then increased to 250 º at a rate of 16ºC/minute, and was held for 20 minutes.

The total plate number of dried kopyor and kopyor extract

A total of 25 grams of dried coconut was inserted into 225 mL of sterile saline solution, and it was shaken overnight on a shaking incubator on 30 °C milk with a speed of 150 rpm. 1 mL of suspension was pipetted, 9 mL of saline solution was added and vortexed, then it was made diluted to 10^{-4}. From 1 mL was pipetted and was put into sterile petridisk, 10 mL *nutrient* media was added until the temperature was 45^{o}C, then was incubated at 37^{o}C for 24-48 hours. The number of colonies was then calculated.

Antibacterial activity test

For microbial test preparation, a representation of gram-negative and positive bacteria was used. Pure culture of test microbes was rejuvenated in nutrient agar sloping media. Setelang was incubated 24 hours at 37°C plus 10 mL of saline solution, shaken with a vortex to release the culture from agar.

The suspension or inoculum was measured by a spectrophotometer and was diluted to 25% transmittance. A total of 5 μL of suspension was inoculated into 8 mL nutrient seed media at 45°C. It was shaken and was poured over the surface of the nutrient base media, which had been compacted in sterile petridisk. The hole was made to use a perforator with a diameter of 0.8 cm and a height of 0.5 cm. Test media was ready for use.

In the testing hole, 50 μL of the test solution both from the powder and kopyor extract was loaded and was dissolved in DMSO with a concentration of 50 mg/10 mL. For comparison, a 200 ppm standard solution was used. Incubation was carried out for 24 hours at 37°C. The observed inhibition and zone diameter were measured (mm).

RESULTS AND DISCUSSION

Characterization of kopyor Cocos nucifera L.

The physical performance of the organoleptic characterization of fresh kopyor and dried kopyor coconut meat, as seen in Table 1 and Table 2.

Table 1: Characteristics of Fresh Kopyor

Parameter	Observation
Form	Soft
Color	White
Smell	Typical coconut
Taste	Sweet

Table 2: Characteristics of dried kopyor resulting from a frozen, dried process

Parameter	Observation
Form	Hard
Color	White yellowish
Smell	Typical coconut
Taste	Sweet

Extraction

Simplicia of dried kopyor meat was extracted by the maceration method. A sample of 272 grams of dried kopyor was added by one Litre of 70% ethanol and was soaked for 24 hours. The filtrate and the residue were separated using a Buchner funnel. The residue was then macerated again twice. All filtrates of extract were collected, the solvent was evaporated using a rotary evaporator until the remaining water phase and then was dried with freeze-drying. The extract was obtained was 62.35 grams and this was equal to extract yield of 22.9 %.

Characterization of kopyor coconut extract

Organoleptic characterization

Kopyor coconut extract after maceration and drying with freeze dryer, the results were obtained and were then organoleptically characterized, as shown in Table 3.

Table 3: Characteristics of the kopyor coconut extract

Parameter	Observation
Form	Hard sticky
Color	Brownish
Smell	Typical coconut

Moisture Content

Results of the moisture content of freeze-dried kopyor meat were 6.42%. The extract did not extract because it was a thick extract and contained high amounts of oil.

Standardization of extracts and characterization

Results of standardization of extracts referred to the Indonesian Pharmacopoeia 5^{th} edition. Standardization was included a loss on drying, total ash content, acid insoluble ash content, water-soluble extract content and ethanol-soluble extract content obtained was shown in Table 4.

Table 4: Standardization of cocos Nucifera meat extract

Parameter of Standardization	Measurement results (% w/w) Average $\pm$ SD
Loss on drying	34.96 $\pm$ 0.30
Total ash content	8.95 $\pm$ 0.10
Levels of acid-insoluble ash	30.94 $\pm$ 2.67
Water-soluble extract content	56.85 $\pm$ 0.97
Level of a soluble extract of ethanol	0.60 $\pm$ 0.20

Saponification Value Test

The results of the saponification value are as follows in Table 5 and Table 6. The saponification value was 56.00.

Characterization of fatty acid content

Characterization of the kopyor extract has been carried out and showed that in the derivatization process, the extract was not stable, produced a blackish-brown liquid. Therefore the process of identification

Table 5: Calculation of standard solution for saponification

m KHP (99.5%); Mr = 204 (gram)	NaOH volume (Vt_1) (mL)	NaOH volume (Vt_2) (mL)	HCl volume (V_{HCl}) (mL)	N HCl (mol / L)
1.0165	10.30	10.45	10.0	0.5025
1.0170	10.30	10.30	10.0	0.4955
			Average N HCl	0.4990

Table 6: Calculation of saponification value

m Sample (gram)	Titration Blank (mL)	Sample titration (mL)	Saponification Number
1.5205	19.80	16.75	56.15
1.5519	19.80	16.70	55.92
		Average	56.00
		x	0.23
		RPD	0.40

with MS GC was not carried out.

Minimum Inhibitory Concentration (MIC) test

MIC test on Staphylococcus aureus

MIC test of standard against *Staphylococcus aureus*, as shown in Table 7.

Table 7: The MIC of standard against Staphylococcus aureus

Concentration (ppm)	Inhibition Zone Diameter (mm) Average ± SD
5.00	19.03 ± 1.89
2.75	11.40 ± 0.54
2.50	10.73 ± 0.71
2.25	9.42 ± 1.51
2.00	8.65 ± 2.19

MIC test on Pseudomonas aeruginosa

MIC test of standard against *Pseudomonas aeruginosa*, as shown in Table 8.

Antibacterial activity test on Staphylococcus aureus,Pseudomonas aeruginosa and Staphylococcus epidermidis

The activity results against Staphylococcus aureus and Pseudomonas aeruginosa were presented in Table 9.

Table 8: MIC of standard against Pseudomonas aeruginosa

Concentration (ppm)	Inhibition Zone Diameter (mm) Average ± SD
5.00	15.70 ± 0.96
2.75	9.23 ± 0.95
2.50	8.10 ± 0.22
2.25	-
2.00	-

Table 9: The diameter of the inhibition zone of extract sand powders against Staphylococcus aureus and Pseudomonas aeruginosa

Samples	Inhibition Zone Diameter (mm)	
	Staphylococcus aureus	Pseudomonas aeruginosa
Cocos nucifera powder		
Positive Control	12.68	10.65
Powder sample 1	14.22	12.25
Powder sample 2	13.37	11.32
Powder sample 3	14.17	12.28
Average	13.92	11.95
Cocos nucifera extract		
Positive Control	12.14	10.88
Extract sample 1	14.25	11.45
Extract sample 2	12.38	12.21
Extract sample 3	13.30	13.27
Average	13.31	12.31

The zone inhibition diameter of extracts and powder samples against the Staphylococcus aureus and Pseudomonas aeruginosa both showed equal activity and significantly showed higher activity compared to the standard solution. This showed the initial information that the powder and extract both have potential as antimicrobials. Antimicrobial activity can be applied to the skin or other route administration. To further strengthen the results for skin diseases, the activity of extracts and powder to the skin or topical disease was then conducted using

skin bacteria such as Staphylococcus epidermidis.

Antibacterial activity against Staphylococcus epidermidis

The antibacterial activity test against Staphylococcus epidermidis bacteria was shown in Table 10.

Table 10: Antibacterial activity against Staphylococcus epidermidis

Sample	Replicati	Staphylococcus epidermidis	
		Inhibition Zone Diameter (mm)	Average (mm)
F1	1	8.90	9.45 ± 0.48
	2	9.65	
	3	9.80	
F2	1	6.50	7.57 ± 1.85
	2	6.50	
	3	9.70	
	Control (-)	5.50	
	Control (+)	6.60	

F1: Cocos nuciferaExtract
F2: Cocos nucifera Powder

The diameter of the inhibitory zone against Staphylococcus epidermidis bacteria showed higher activity results compared to the standard solution. The kopyor Cocos Nucifera L. extract and powder demonstrated that both had potential as antimicrobials, especially to skin diseases.

CONCLUSION

Cocos nucifera meat extract has been characterized compared to dried powder Cocos nucifera. The antibacterial activity results showed the potential of cocos Nucifera extract against *Staphylococcus aureus, Pseudomonas aeruginosa and Staphylococcus epidermidis* bacteria. This potential active agent can be suggested for further evaluation for topical disease application or other route administration.

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