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RESEARCH ARTICLE

The Effect of Freeze-Drying Procedures on Frangipani Sap Chloroform Extract Quality

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ABSTRACT:

The water content in the frangipani sap enormously influences the product of the extraction process of natural materials. Before extraction, water content on the herbal material based sap is reduced by using the drying process. This study aimed to analyze the impact of water content reduction through freeze and non-freeze dry in the quality chloroform extract of frangipani sap. This research was designed into two groups to reduce frangipani sap water content through freeze-drying and without freeze-drying. Both groups were extracted by maceration method and chloroform solvents. The result of extraction was seen the physical quality and phytochemical screening through the Thin Layer Chromatography method (flavonoids, anthraquinone, polyphenols, alkaloids, terpenoids, and triterpenoids). Furthermore, the color intensity assay is produced from the Thin Layer Chromatography assay results and then carried out statistical tests. The results of this study are that without the freeze-drying group there is a fungus in the storage period. Both groups do not contain active ingredients flavonoids, anthraquinone, and polyphenols but contain alkaloids, terpenoids, and triterpenoids. The results of the color intensity test on the active ingredients contained showed that the freeze-drying group was higher and there were statistically significant differences in values. The results of this study indicated that chloroform extract of frangipani extract by freeze-drying showed higher quality than non-freeze drying. In the freeze-drying group, it can be found that there were no fungi and had the right color intensity. However, this study requires further study to know the compound properties quantitatively.

KEYWORDS: Frangipani sap, freeze-drying, physical quality, phytochemical screening, color intensity.

INTRODUCTION:

Extensive efforts have been made to investigate the alveolar bone remodeling manipulation agent in the acceleration of tooth orthodontic movement by using herbal as Frangipani sap. The previous study described the sap and leaves of Plumeria alba presented the ability in the acceleration of tooth movement, antibacterial, and non-toxic agents[1,2,3]. Nowadays, the usage of herbal becomes a trend in research either as medicine or treatment, although the application presented not optimal due to the inappropriate extraction process[4,5].

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Water content in the sap enormously influences the product of the extraction process of natural materials. The impacts of excessive water content are the increased heating temperature, the amount of solvent, physical endurance during storage, and not maximal active compound[6,7]. Water content represents the amount of water in materials stated in percentage. However, the water content presents the essential food properties due to may influence food performance, texture, and taste. Moreover, it determines food freshness and durability, in which excessive water content triggers the propagation of bacteria, mold, and yeast[8].

Before extraction, water content on the herbal material based sap is reduced by using the drying process[9]. One of the drying methods is freeze-drying. Freeze drying is a technique that makes it possible to dehydrate all heat-sensitive materials. This technique is a multi-stage procedure to stabilize the material through four main procedures such as freezing, sublimation, desorption, and finally storage[10]. It will produce water vapor from ice. The advantage of this method is to reduce active compound destruction, prevent material shrinkage, preserve taste, and not induce smell[11,12]. This is supported by Marin [13] stated that freeze-drying is a drying process this is very appropriate for the conservation of biological products. Compared to other drying processes, Freeze-drying is taken into consideration as a reference protocol for the manufacture of high-quality dehydrated products. The direct transition of water from solid to vapor (sublimation), without the liquid phase, helps keep most of the residences of the preliminary raw material's which include appearance, shape, taste, color, and flavor.

Aforementioned, this study aimed to analyze the impact of water content reduction through freeze and non-freeze dry in the quality chloroform extract of frangipani sap.

MATERIALS AND METHODS:

Frangipani Sap:

Identification and Selection of Frangipani Plant:

The sap was collected from frangipani plant species of *plumeria acuminata ait*. The plants were previously registered at the Plant Taxonomy Laboratory of the Biology Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, by showing the examples of flowers, leaves, and photos of the trees.

Frangipani plants are randomly selected with the following criteria (1) Growing in Jember Regency, east java, Indonesia and surrounding areas, (2) Trees are old enough with brown bark markings with a minimum stem circumference of 45cm measured in sections 120cm away from the ground.

The Procedure of Frangipani Sap Collection:

Frangipani plants that have been selected and according to the criteria are marked and documented. Frangipani sap is obtained through tapping tree trunks (adoption of rubber plants and other gummy wood plants). Tapping performed at 04.00 to 07.00 am. The trunk that will be tapped for sap is a tree trunk which is 120cm from the ground. Tree trunks are injured using a sharp tapping knife with a depth of 1-2mm (without damaging the cambium), as long as 30 cm from top left to bottom right diagonally at an angle of 30-40° (the area of injury is marked first). Make the grooves of the wound level vertically down at the end of the injury along the 5cm. A gutter is placed at the end of the vertical wound to drain the sap that has come out. Glass bottles are placed to hold the sap under the chamfer. The sap is needed as much as 1000ml, if in one tree cannot produce the sap to the desired amount, then the tapping is done on other trees to produce 1000ml of sap. The method of collecting sap is combined with cutting the trunk of a tree which is about 50cm from the tip of the trunk, the sap that comes out of the trunk that has been cut or attached to the trunk is collected using a container made of glass. After getting 1000ml of sap, the sap is filtered to be free of impurities, the container is tightly closed to avoid the sun and moisture. During storage, the sap is collected in a glass container and put in an iced box and the storage is placed in a refrigerator at 4°C.

Freeze-Drying Frangipani Sap Extraction Procedure:

The 100 gram Frangipani sap was put in the sample tray of the freeze dryer. The tray put in a freeze dryer, and then the cover was closed by air-tight. Freeze dryer was switch on and set in drying for 60 hours on -80ºC and pressure are set as 1 atm. After 60 hours and the vacuum pressure stopped, the tray was put off, and so was the sample. The dry frangipani sap was mashed by blender, and the next, it was sifted by #60 mesh.

Dry frangipani sap powder, which result of #60 mesh was put in macerator. It was added chloroform five times of powder weight (gram, and it was stirred. The maceration process needed three days. For important procedures, during the maceration process, the cover of the macerator was closed, and two times a day, it was stirred. After maceration, the macerate was sifted by the Buchner funnel. Then, it was concentrated by *rotary evaporator* in 40ºC and 150rpm till getting lumpy extract chloroform free. The chloroform extract of frangipani sap was put in the tray and was kept in the refrigerator (4°C).

The water content analysis is carried out directly, based on weighing material weight. The difference between fresh material weight and dry weight is the moisture content in the material being examined. Water content is expressed in percent. The weight difference before and

after drying is the amount of water evaporated.

Without Freeze-drying Frangipani Sap Extraction Procedure:
Frangipani sap was added chloroform five times of sap weight (100gram), and it was stirred. The maceration process needed three days, and two times a day, it was stirred in closed macerator. After maceration, the macerate was sifted by the Buchner funnel. Then, it was concentrated by *rotary evaporator* in 50ºC by 150rpm till getting lumpy extract chloroform free. The chloroform extract of frangipani sap was put in the tray and was kept in the refrigerator (4°C).

Taking Photo of Frangipani Sap Extraction Results:
This procedure was carried out on the results of frangipani sap extract and test plates on the TLC assay results. This procedure uses DSLR camera (Nikon D7200, Nikon AF-S NIKKOR 85mm f/3.5G ED DX VR Micro Lens, Macro LED ring flash).

Thin-Layer Chromatography (TLC) Assay of Frangipani Sap Chloroform Extract:
TLC examination aims to conduct an initial screening test of the active ingredients of frangipani extract in both groups. The active ingredients detected were flavonoids, anthraquinone, polyphenols, alkaloids, terpenoids, and triterpenoids[14,15]. The presence of active ingredients detected is obtained from stains found on the plates.

Flavonoid Compound:
The extract (0.1g) was put in 1ml n-hexane and repeatedly stirred until the extract was colorless. The residue was dissolved in some drops of ethanol, and then it was dropped on thin-layer chromatography (silica gel 60 F254). After that, it was eluted by the mobile phase of butanol-glacial acetic acid-water (4:1:5). It was subsequently sprayed stain viewer of borate citric. The intensity yellow color indicated a flavonoid presence.

Anthraquinone Compound:
The extract (0.1g) was added some drops of ethanol until the extract was dissolved. Then it was dropped on thin-layer chromatography (silica gel 60 F254). After that, it was eluted by a mobile phase of toluene-ethyl acetate-acetic acid (75:24:1). It was subsequently sprayed stain 10% KOH reactor in methanol. Yellow, tawny, purplish-red, or purplish-green indicated anthraquinone.

Polyphenol Compound:
The extract (0.1g) was added in 3ml hot water, and then it was stirred until room temperature. After that, it was added two drops of 10% NaCl, and it was subsequently stirred and sifted. Next, it was dropped on thin-layer chromatography (silica gel 60 F254). After that, it was eluted by a mobile phase of toluene-acetone-formic acid (6:6:1). It was subsequently sprayed stain 10% FeCl3 reactor. The black color indicated polyphenol.

Alkaloid Compound:
The extract (0.1g) was added in 2ml 2N HCl. Then it was heated on a water bath for 2-3 minutes while stirring. After it was cold, it was added 0.1gr NaCl. It was subsequently stirred and sifted. The filtrate was added 2ml 2N HCl, 28% NH4OH until it was dissolved, and to be alkaline. The filtrate was extracted in 5ml chloroform-water free. Then, it was evaporated until it was dry and dissolved some drops of methanol. Next, it was dropped on thin-layer chromatography (silica gel 60 F254). After that, it was eluted by a mobile phase of ethyl acetic-methanol-water (9:2:2). It was subsequently sprayed stain Dragendorf reactor. The orange color indicated alkaloids.

Terpenoid Compound:
The extract (0.1g) was added to some drops ethanol and stirred until dissolved. Next, it was dropped on thin-layer chromatography (silica gel 60 F254). After that, it was eluted by a mobile phase of n-hexane-ethyl acetic (4:1). It was subsequently sprayed stain anisaldehyde sulphuric acid reactor. The red-purplish indicated terpenoids.

Triterpenoid Compound:
The extract (0.5g) was added to 2ml 2N HCl. It was boiled and covered by funnel containing wet cotton for 2 hours for saponin hydrolysis. After it was cold, it was neutralized by ammonia. Next, it was extracted by 3 ml n-hexane and evaporated until it was 0.5ml. Next, it was dropped on thin-layer chromatography (silica gel 60 F254). After that, it was eluted by a mobile phase of n-hexane-ethyl acetic (4:1). It was subsequently sprayed stain anisaldehyde sulphuric acid reactor. After that, it was heated at 115°C for 5-10 minutes. The red-purplish indicated triterpenoids.

Color Intensity Assay to The Result of TLC:
The picture of thin-layer chromatography assay in chloroform extract frangipani sap was taken with a DSLR camera (Nikon D7200, Nikon AF-S NIKKOR 85mm f/3.5G ED DX VR Micro Lens, Macro LED ring flash).

The measurements of color intensity were performed using the ImageJ 1.52i software (Wayne Rasband National Institute of Health, USA). The results of the color intensity test were analyzed statistically using the SPSS program (IBM) with a significance level of 0.05 ($p = 0.05$) and a reliability level of 95% ($\alpha = 0.05$).

RESULTS:
The Physical Quality of Frangipani Extract:
Frangipani extract which documented by the camera was taken in storage for 2 weeks at 4℃ temperature as shown in Figure 1.

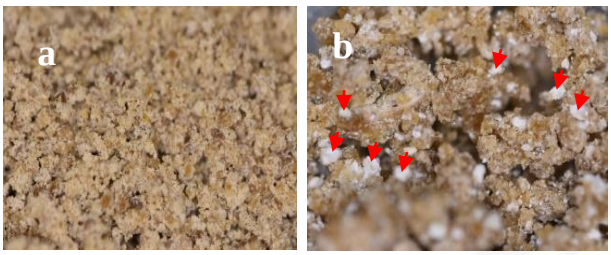

Figure 1. The chloroform extract of frangipani sap (taken by Nikon d7200 DSLR camera)
a. with freeze-drying group (FD); b. without freeze-drying group (WFD); red arrow = fungus

Figure 1 described that the result of frangipani sap extract without freeze-drying group (WFD) presented better quality than with freeze-drying group (FD). Based on this fact, frangipani sap extract without freeze-drying group indicated fungi evenly distributed on frangipani sap extract.

The weight of frangipani sap in the FD group after the drying process was 24.8 gr grams of 100grams of fresh sap. Based on the weight ratio of fresh and dried latex, the moisture content is 75.32%. In other words, the amount of water evaporated through the freeze-drying process is 75.32%.

Result of TLC Assay of Frangipani Sap Chloroform Extract:

Frangipani sap extract was analyzed by its active compound, such as flavonoid, anthraquinone, polyphenol, alkaloid, terpenoids, and triterpenoids by TLC. The result was showed in Figure 2.

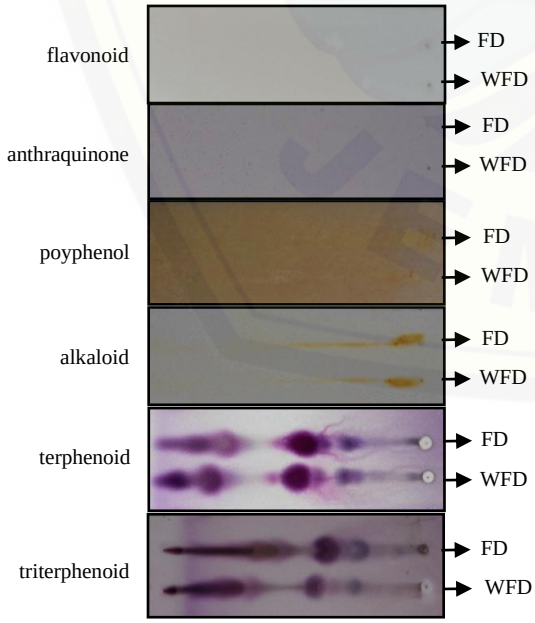

Figure 2. Thin-layer chromatography of frangipani extract in both extraction methods

Figure 2 described the result of the Thin-layer chromatography assay. The test results were that the flavonoid, anthraquinone, and polyphenol compound tests in both groups did not show the appearance of stains on the test plate. Alkaloid compound test in both groups contained orange stain appearance with different intensity on the test plate. The Terpenoid and Triterpenoid compound tests showed red-purplish colored stains with different intensities on the test plate. Based on the stains, chloroform extract of frangipani sap in both groups did not contain flavonoid, anthraquinone, polyphenol; however, it contained alkaloid, terpenoid, and triterpenoids.

Color Intensity Assay in TLC Assay Result:

This assay used software ImageJ 1.52i of photographs taken by DSLR camera. This assay represented an initial approach to know the active compound in frangipani sap extract by two different drying methods. The data were presented in Table 1.

Table 1. Color intensity Assay

No	Compounds	Result	
		TLC	Color Intensity (pixel)
1	Flavonoid		
	- FD	Negative	n/a
	- WFD	Negative	n/a
2	Anthraquinone		
	- FD	Negative	n/a
	- WFD	Negative	n/a
3	Polyphenol		
	- FD	Negative	n/a
	- WFD	Negative	n/a
4	Alkaloid		
	- FD	Positive	111.45 ±12.65*
	- WFD	Positive	107.42 ± 12.59
5	Terpenoid		
	- FD	Positive	137.23 ± 12.71*
	- WFD	Positive	135.39 ± 12.73
6	Triterpenoid		
	- FD	Positive	139.73 ± 12.69*
	- WFD	Positive	123.04 ± 12.74

Note: * the value is a statistically significant difference compared to WFD group

Table 1 describe that compounds that were stated as positive in the FD method had a higher value than in the WFD method. The results of statistical tests that have been carried out are that the FD group has a significantly different value ($p < 0.05$) when compared to the WFD group. This result proved that FD methods were more appropriate to maintain the active compound than WFD during the extraction process.

The Summary of Result:

Based on the results above, the summary of the result described in Table 2.

Table 2. The summary of assay of frangipani sap extract in both methods

No	Characteristic	Result		Explanation
		FD group	WFD group	
1.	Extraction material			Water content: 75.32%
	Form of extraction material	Powder (freeze-drying process)	Fresh sap	
	Amount of extraction material	24.8 gr	100 gr	
2.	Amount of solvent during the extraction process	Minimal (123.38 ml)	Most (500 ml)	
3.	Drying Periods	60 hours	-	
4.	Extraction process periods			
	Maceration time	72 jam	72 jam	
	Evaporation process time Evaporation process temperature	1 jam 40°C	3 jam 50°C	
5.	Fungi presence	no	yes	2 weeks of storage at 4 °C
6.	Active compound absent	Flavonoid, polyphenol, anthraquinone	Flavonoid, polyphenol, anthraquinone	Result of TLC assay
7.	Active compound presence	Alkaloid. Terpenoid, triterpenoid	Alkaloid. Terpenoid, triterpenoid	Result of TLC assay
8.	Color intensity assay	higher	Lower	Positive of TLC assay

Note: FD (freeze-drying group), WFD (without freeze-drying group), TLC (Thin Layer Chromatography)

DISCUSSION:

The drying process of frangipani sap represents the water reduction process in some materials until the microorganism growth was absent. The absence will inhibit the activities, including the decomposition process. The more water content in materials, the faster the decomposition process[16]. Therefore, the dry materials have storage time longer without nutrition content change than materials extracted without the drying process. Beside it, materials size influences the drying process. The smaller the size of the material, the faster the drying process, and the little water content, both in natural or modification drying process[17]. The function of water is as material, which may be dispersing the compound in materials. The water content influences physical characteristics (stiffness and drought), physic-chemical, and chemical changes (enzymatic process, microbiology destruction, and enzymatic alteration). Water content in the material before extraction influences the final result either during the extraction process[18].

Water content influences food durability both in wet and dry foods. The function of water is as material, which may be dispersing the compound in materials. The water content influences physical characteristics (stiffness and drought), physic-chemical, and chemical changes (enzymatic process, microbiology destruction, and enzymatic alteration). The high water content in foods causes short of storage periods because it triggers fungi growth[19].

Based on the methods and table 2, the extraction cost was cheaper than WFD due to the FD method required less chloroform solvent than the WFD method during the extraction process. The evaporation temperature in the FD method aimed to reduce the solvent was lower than WFD because the high water content in the WFD method needs high temperatures. This temperature may destroy the active compound of materials, which is thermosensitive[20]. The water content causes an increase in the evaporation temperature and process period[21]. The drying process by high temperature may trigger the destruction of the color, texture, flavor, and active compound of materials, although it may accelerate the extraction process[9,22].

This study was as Sembiring[23] study which stated that the longer the drying time, the lower the quality of the material because of long heat exposure. The result described the different methods might not influence the active compound concentration, yet it could affect the water content. The drying process using the freeze dryer was safer form active compound degradation than the non-freeze dryer because the temperature is low. The fungi grow readily when the water content in the extract is high, causing the poor quality of extract. Das *et al.*[24] stated that drying using oven heating had high economic value, although it could reduce the antioxidant capacity. This result is supported by Mishara *et al.*[25] and Retno. *et al.*[26] who states Freeze-drying is more effective drying method than the usual process to improve the stability of bioactive products, and Simmy *et al.*[27,28] also support this result by stating that although the Freeze-drying method is expensive, this method has an important role in drying sensitive materials for better stability of bioactive components.

This study showed that the color intensity of active compounds in the FD group was more significant than the WFD group. It indicated the high color intensity might provide a highly active compound. Reyes *et al.*[29] and Vuthijumnok *et al.*[30] stated that blueberry extracted by the FD method presented high phytochemical compound and antioxidant activity significantly. However, Aji *et al.*[11] stated that the long duration of the extraction process immensely affects the extraction product. The increased process period will enhance yield

value, as the long extraction period will enhance solvent penetration into raw materials. The materials component solubility is linear slowly to the increased time, although after the optimum period, the amount of collected compound will decrease. Therefore the composition containing materials is limited, and the used solvent has a limitation in dissolving the materials. Although extraction periods are extended, the solute has been absent.

CONCLUSION:

The results of this work indicated that chloroform extract of frangipani extract by freeze-drying showed higher quality than non-freeze drying. In the freeze-drying group, it can be found that there were no fungi and had the right color intensity. However, this study requires further study to know the compound properties quantitatively.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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