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Research Article

Formulation and antibacterial activity of frankincense sap extract deodorant spray (*Styrax benzoin*)

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Keywords

Bacteria
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Abstract

Deodorant is a cosmetic preparation used by the wider community to control and reduce body odor. Deodorant act as antibacterial and are able to reduce the excretion of sweat in the armpits. Frankincense is a plant that contains flavonoids and tannins can act as antibacterial. This aim of this study was to determine the effectiveness of frankincense sap extract as an antibacterial in deodorant spray preparation. Frankincense sap powder is macerated with ethanol to obtain frankincense sap extract. Deodorant formulations are prepared from frankincense sap extract in various concentrations (5%, 10%, 15%, 20%, 25%) and combined with cosolvent, emollient, and solvent. Tests carried out include antibacterial testing against *Pseudomonas aureugenisa* and *Staphylococcus epidermidis* bacteria, organoleptic test, pH test, viscosity test, and irritation test. The test results showed that antibacterial testing gave the highest inhibitory diameter of 2.63 mm and 5.35 mm which was exhibited by formulation 5.

Introduction

Indonesia is a tropical country that is always illuminated by the sun, so the average air temperature is high enough to trigger sweat production. Excessive sweat production can cause problems, especially unpleasant body odor (Veranita et al. 2021). Sweat produced from the excretory process that occurs in the body. The excretory system is needed to maintain body temperature, where the body will remove toxins through sweat, urine and the process of respiration (Aji and Ashadi, 2019). Eccrine and apocrine glands are sources of sweat. Apocrine glands contain fat and protein, which cause unpleasant odors if broken down by bacteria, this odor is known as body odor (Maftuhah et al. 2015).

The use of soap and water as body wash at bath time is relatively less effective to prevent body odor. So that several other alternative actions can be done, such as using anti-body odor cosmetic preparations (deodorant). Deodorant is a product that serves to reduce or mask body odor. Deodorant are also antiprespirant, because deodorant act as antibacterial and are able to reduce sweat excretion in the armpits (Meitasari et al. 2015). Deodorants show antimicrobial activity by reducing the body's pH which can inhibit the growth of microorganisms that produce unpleasant odors (Egbuobi et al. 2012).

Natural-based deodorants are hard to find and have not been produced on a large scale on the market. Indonesia is rich in flora sources and many of them can potentially be deodorants, one of which is frankincense. Frankincense is one of the nutrient-rich plants that grows in Indonesia, especially North Sumatra. According to Jayusman (2014) there are 3 types of frankincense in North Sumatra, namely toba frankincense (*Styrax parallelloneurum* PERK), durame frankincense (*Styrax benzoine* Dryand), and feather frankincense (*Styrax benzoine* var hiliferum).

Frankincense (*Styrax benzoin*) is a non-timber forest product with the genus Styracaceae. The level of phenolics and flavonoids contained in this species are a benchmark for antibacterial activity possessed. According to Suparto et al. (2019) frankincense (Styrax benzoin) contains phenolic and flavonoid derivative

compounds, namely 1-(2,4-dihydroxyphenyl)-2-(4-hydroxyphenyl) propane-1-on, chromone, 5,7-dihydroxy-2-(4-hydroxyphenyl)-6,8-dimethyl-2,3-dihydroxychromon-4-on, 5,7-dihydroxy-2-(4-hydroxyphenyl)-8-(3 methylbut-2-enyl)-2,3-dihydrochromon-4-on, and prolifisin A.

Frankincense is one of plant commodities in North Sumatera that has been used as medicinal plants to treat infection and illness. Isolates on the leaves, bark and roots of frankincense showed considerable antibacterial activity against *Staphyloccocus aureus*, *Escherichia coli* and *Streptococcus mutans*. Frankincense leaf isolate has an inhibitory zone of 10 mm in *S. mutans*, 15 mm in *S. aureus*, and 8 mm in Escherichia coli. Frankincense bark isolate has an inhibitory zone of 10 mm in *S. mutans*, 7.50 mm in *S. aureus*, and 8.45 mm in Escherichia coli. While frankincense root isolate has an inhibitory zone of 9 mm in *S. mutans*, 13 mm in *S. aureus*, and 10 mm in *E. coli* (Siregar et al. 2019). Agustian (2010) have proven that 500 mg/ml ethanol extract of frankincense leaves in gel preparations has an antibacterial effect on *Propionibacterium acne* and *Staphylococcus epidermidis* with inhibitory zone diameters of 14.02 mm and 21.73 mm respectively. Based on the description above, research was carried out on the formulation of deodorant spray preparations of frankincense gum extract (*S. benzoin*) as antibacterial.

Method

Sample and Materials

Frankincense gum powder from Parsoburan area, ethanol 96%, propylene glycol (PG), glycerin, dragendrof reagent, Magnesium powder, Hydorgen chloride (HCl), Iron (III) chloride ($FeCl_3$) 1%, nutrient agar (HIMEDIA), Dimethyl sulfoxide (DMSO), chloramphenicol (Oxoid), physiological Sodium chloride (NaCl) 0.9%, analytical balance, set of glassware, split funnel, ostwald viscometer 2 mL (pyrex), maceration bottle, aluminum foil, rotary evaporator, magnetic stirrer, hotplate, bunchner, vacuum pump, autoclave, petri dish, ose needle, disc paper 6 mm (oxoid), micro pipette 100-1000 ɥL (eppendorf).

Extraction

Frankincense sap powder measuring 80 mesh weighing 250 g was extracted by maceration or soaking method using 96% ethanol solvent with a ratio of 1 : 2. Extraction was carried out for 3 x 24 hours at room temperature and remaceration was carried out on days 2 and 3 (Chairunnisa et al. 2019). Then the entire filtrate is filtered with filter paper using a buncner filter and a vacuum pump. The filtrate is separated and concentrated on the rotary evaporator at the boiling point of the solvent within 4-5 hours (Savitri et al. 2017 modified).

Identification of Secondary Metabolites

Examination of the content of alkaloid compounds was carried out with Dragendroff reagents, flavonoids with Mg and HCl(p) powder (Wahid and Safwan, 2020), tannin compound content was carried out with 1% $FeCl_3$ (Susanti et al. 2021) and saponin content tests were carried out by shaking in distilled water and HCl (Susanti et al. 2021).

Formulation of Deodorant Spray of Frankincense Sap Extract

The frankincense deodorant spray was made in 5 different concentrations. This was aimed for determination of effective frankincense concentration to exhibit the antibacterial activity. Table 1 showed the different concentration of frankincense in all 5 formulas.

Table 1. Formulation of Deodorant Spray of Frankincense Sap Extract

Material	Function	Formula (%)				
		F1	F2	F3	F4	F5
Extract (b/v)	Active substances cosolvent	5	10	15	20	25
Propylene glycol (v/v)	Emollient	5	5	5	5	5
Glycerine (v/v)	Solvent	10	10	10	10	10
Ad ethanol 96% (v/v)		100	100	100	100	100

Antibacterial Activity Test of Deodorant Spray

Sterile NA agar media is poured as much as 20 mL in a petri dish and allowed to solidify at room temperature. Then 100 L of test bacterial suspension is dripped into the medium and leveled. The ingredients, bases, and various deodorant spray formulations were dripped onto sterile disc paper, then placed in a petri dish

containing solid agar media spiked with test bacteria, DMSO (negative control), and chloramphenicol (positive control). Then incubated at a temperature of 37 for 24 hours. After 24 hours, the diameter of the clear inhibitory zone formed was calculated using a caliper (Rizqiyana et al. 2014).

Organoleptic Test

Organoleptic tests are carried out by observing the color, shape, odor, and taste of the preparation. Pipette 5 ml of product into the vial and observe the color and shape, for odor is done by breathing air over the vial. Sensory tests are performed after formulation, and reevaluated on day 7.

Acidity or pH Test

Acidity or pH testing is carried out with the help of universal pH indicator. Measurements are made by dipping pH paper into the product and adjusting the formed color to the available pH susceptible colors. pH testing is performed after formulation, and re-evaluated on day 7.

Viscosity Test

Viscosity testing is performed with an Ostwald viscometer, where viscosity is calculated using an aqueous reference solution by measuring the time it takes for a liquid to pass through two predetermined points in the capillary tube.

Table 2. Interpretation of irritant test results

Reaction	Result
No change	(-)
Red skin	(+)
Red and itchy skin	(++)
Swollen skin	(+++)

Irritation Test

Irritation tests are also called skin sensitivity tests. Deodorant spray formulations should be checked for irritating or sensitive to the skin (Voigt, 1995). The tests were conducted directly on 10 volunteers who did not have skin diseases or allergies, both men and women. The test is carried out using a spray test method where the test product is sprayed onto the inner arm. Symptoms are observed after 6 hours. The following Table 2 showed the interpretation of irritant test.

Results and Discussion

Extraction and Identification of Secondary Metabolites

Flavonoid compounds and phenolic compounds are obtained by extraction, which is a filtering process from the source. Conventionally flavonoids are separated from the plant matrix by methods requiring high amounts of solvent, long separation times, and low recovery, and optimized with appropriate solvent selection (Hakim and Saputri, 2020).

This study used the maceration method with 96% ethanol as a solvent. Maceration is carried out by immersing the sample in organic solvents. The soaking process causes organic solvents to penetrate the cell wall and enter the cell cavity containing secondary metabolites, secondary metabolites will dissolve and because of the difference in concentration between the solution inside and outside the cell, the more concentrated solution will come out of the cell carrying secondary metabolites. This process continues to repeat until there is a balance of concentration between the solution inside and outside the cell (Nugraha et al. 2017).

Ethanol solvent is used as a solvent because it is polar so that it can attract phenol compounds, flavonoids, tannins, and saponins. Ethanol is relatively non-toxic compared to acetone and methanol, is cheap, can be used in various extraction methods, and is safe for extracts to be used as drugs (Hakim and Saputri, 2020). Beside that, frankincense has partial solubility properties because the organic acid content it has is not ionized with water so that water/aquetest cannot be used as a solvent, effective in producing the amount of active / yield ingredients (Simatupang et al. 2021). A total of 250 grams of frankincense gum powder was macerated with 96% ethanol solvent, the total solvent used was 1500L, the extract obtained was concentrated with a vacuum rotary evaporator obtained a viscous extract with a yield of 83.88%.

The content of secondary metabolites in the ethanol extract of frankincense sap was identified to see the content of compounds that can act as antibacterials. Alkaloid compounds are able to interfere with the constituent components of peptidoglycan contained in bacterial cells so that the cell wall layer is not formed intact and causes cell death. This is what makes alkaloids as antibacterial agents (Haryati et al. 2015). The results of the identification of secondary metabolites are presented in Table 3.

Table 3. Results of identification of secondary metabolites

Compound Classes	Result	Information
Alkaloids	+	Orange color formed
Flavonoids	+	Formed reddish-brown color
Tannins	+	Formed blackish-green color
Saponins	+	Formed foam

+ : Contains secondary metabolite compounds;
- : Does not contain secondary metabolite compounds

Formulation and Bacterial Activity Test of Deodorant Spray Formulation

Propylene glycol and glycerin were homogenized at 500 rpm at 25 and 4 mL of 96% ethanol (base) was added. Frankincense sap extract was weighed according to a predetermined weight, and dissolved into 96% ethanol. Solution of frankincense sap extract is mixed into the base. 96% ethanol is added until the total volume reaches 20 mL and homogenized.

Propylene glycol was chosen as a cosolvent because it can help dissolve glycerin, has low toxicity, and has a function as a plasticizer that can help deodorant spray function optimally because it helps the product bind to the skin (Rowe et al. 2009) as well as increasing the stability of the preparation (Susanti et al. 2021). Glycerin is used as an emollient to give a soft impression to the preparation, because the sap of frankincense has a sticky texture, and leaves a rough texture after rinsing with water. While 96% ethanol acts as a solvent to dissolve frankincense into other materials and facilitate spraying preparations and facilitate the drying process because 96% ethanol is volatile.

Table 4. Antibacterial test results of deodorant spray ingredients

Treatment	Average Resistance Zone (mm) ± SD	
	Pseudomonas aeruginosa	*Staphylococcus epidermis*
Chloramphenicol(Control +)	12.73 ± 1.05	13.17 ± 0.86
DMSO (Control -)	0 ± 0.00	0 ± 0.00
Propylene glycol	0 ± 0.00	1.06 ± 0.24
Glycerine	0 ± 0.00	2.26 ± 0.77
Ethanol 96%	0 ± 0.00	1.83 ± 1.10

Table 5. Antibacterial Deodorant Spray Test Results

Treatment	Average Resistance Zone (mm) ± SD	
	Pseudomonas aeruginosa	*Staphylococcus epidermis*
Chloramphenicol (Control +)	11.35 ± 0.02	14.70 ± 1.59
DMSO (Control -)	0 ± 0.00	0 ± 0.00
F1 (extract 5%)	1.92 ± 0.12	4.0 ± 0.47
F2 (extract 10%)	2.00 ± 0.13	4.63 ± 0.88
F3 (extract 15%)	2.45 ± 0.39	5.15 ± 0.12
F4 (extract 20%)	2.51 ± 0.53	5.08 ± 0.30
F5 (extract 25%)	2.63 ± 0.75	5.35 ± 0.78

Antibacterial activity testing of frankincense gum extract deodorant spray preparation (*Styrax benzoin*) on *P. aeruginosa* and *S. epidermis* bacteria gave the results of an inhibitory zone around the disc paper in each formulation with an average inhibitory power of ± 1-5 mm. Davis and Stout in Ariyani et al. (2018) states that the strength of bacterial inhibition is seen from the size of the diameter of the given inhibitory zone. Resistance is said to be weak when it has a diameter of <5 mm. If the diameter has 5-10 mm, it has a medium inhibitory ability. While a diameter of 10-20 mm is categorized as strong inhibitory, and >20 is categorized as very strong.

Based on both Table 4 and Table 5 above, it can be seen that the frankincense gum extract deodorant spray has a weak bacterial inhibitory ability against the two test bacteria. Some factors that can cause the diameter of

the resulting inhibitory zone also decreased when compared to the diameter of the inhibitory zone of the frankincense sap extract itself. Decreased performance of antibacterial activity of extracts can be caused by the addition of other ingredients when making deodorant spray.

The results of antibacterial activity tests also show that deodorant spray is more sensitive to gram-positive bacteria. This can be seen from the size of the resulting inhibitory zone. *S. epidermis* has a wider diameter of inhibition zones than *Pseudomonas aeruginosa*. This difference can be caused by differences in the structure and constituent components of the bacterial cell wall (Nurhamidin et al. 2021). The peptidoglycan layer present in the cell wall of gram-positive bacteria is thinner and the constituent components of the cell wall are complex because it has an additional outer membrane layer, while in gram-negative bacteria it is thicker. These bacteria have complex cell wall constituent components because they have an additional outer membrane layer, so it is easier to penetrate the gram-positive cell wall than gram-negative (Octaviani et al. 2019).

Organoleptic Test

Organoleptic testing of deodorant spray formulations is an assessment carried out using the help of the sense of sight and smell. This test aims to determine the characteristics of the five formulations of frankincense gum extract deodorant spray after preparation and after a storage period of 7 days. The organoleptic deodorant spray test results are listed in the following Table 6.

Table 6. Organoleptic Test Results of Deodorant Spray Products

Test	Formula	Organoleptic by storage time	
		Day 7	Day 7
Form	F1	Liquid	Liquid
	F2	Liquid	Liquid
	F3	Liquid	Liquid
	F4	Liquid	Liquid
	F5	Liquid	Liquid
Color	F1	Brownish yellow	Brownish yellow
	F2	Brownish red	Brownish red
	F3	Brownish red	Brownish red
	F4	Brownish red	Brownish red
	F5	Blackish brown	Blackish brown
Smell	F1	Typical frankincense	Typical frankincense
	F2	Typical frankincense	Typical frankincense
	F3	Typical frankincense	Typical frankincense
	F4	Typical frankincense	Typical frankincense
	F5	Typical frankincense	Typical frankincense
Taste on the skin	F1	Soft and cool on the skin	Soft and cool on the skin
	F2	Soft and cool on the skin	Soft and cool on the skin
	F3	Soft, cold and slightly sticky to the skin	Soft, cold and slightly sticky to the skin
	F4	Soft, cold and sticky on the skin	Soft, cold and sticky on the skin
	F5	Soft, cold and sticky on the skin	Soft, cold and sticky on the skin

pH Test

Each preparation of deodorant spray gum frankincense extract is tested for pH to determine the acidity of the formulation that has been made. This test is closely related to the nature of the resulting irritation. Products that have a pH above the normal pH of the skin will potentially cause dry skin. If the pH of the preparation is at the normal pH of the skin, it is likely to potentially cause irritation (Ervianingsih and Abd, 2019). All five formulations have a pH value of 4. Thus, the pH produced by the five deodorant spray formulations has met underarm pH standards. Zahara (2018) that the pH of underarm skin is different from the physiological pH of the skin. Underarm skin has a pH of 3.9-4.2 while physiological skin has a pH of 4.5-6.5.

Viscosity Test

Viscosity measurement is carried out with the help of the Ostwald Viscometer tool by comparing the viscosity of the preparation with the viscosity of water (Riyanta and Febriyanti, 2018). The viscosity values of the five formulations can be seen in the following Table 7.

The viscosity of a solution is directly proportional to its concentration. If the solution concentrates high, then its viscosity is high as well. This happens because the concentration of the solution indicates the amount

of solute per unit volume (Lumbantoruan and Yulianti, 2016). This is what causes the five deodorant formulations to have different viscosities.

Table 7. Deodorant spray viscosity test results

Formula	Viscosity (cP) ± SD
F1	1.4312 ± 0.23
F2	1.8190 ± 0.23
F3	2.1316 ± 0.88
F4	2.6936 ± 0.23
F5	2.8181 ± 0.00
Distilled water	0.8904 ± 0.00

F5 has the greatest viscosity because the concentration of frankincense sap extract on F5 is also the largest. Thus, F1 and F2 spray deodorants are the most optimal deodorants, because their viscosity is close to the viscosity of aquades.

Irritation Test

Irritation tests are made to determine sensitivity and anticipate side effects on the skin. Irritation tests were conducted on 10 panelists who did not have allergies or skin diseases. The test results have been attached in Table 8.

Table 8. Irritation test results of deodorant spray frankincense sap extract

Formulasi	Panelists' Reactions									
	1	2	3	4	5	6	7	8	9	10
F1	-	-	-	-	-	-	-	-	-	-
F2	-	-	-	-	-	+	-	-	-	-
F3	-	-	-	-	-	+	-	-	-	-
F4	-	-	-	-	-	+	-	-	-	-
F5	-	-	+	-	-	+	-	-	-	-

Information: - : No change; + : Red skin; ++ : Red and itchy skin; +++ : Swollen skin

The test results showed that there was 1 volunteer who experienced irritation in the F2-F5 formulation preparation and 1 volunteer experienced irritation in the F5 formulation preparation. The reaction caused is reddened skin in the area sprayed with deodorant spray. The reaction caused is irritation of erythema. Erythema is a primary irritation that gives an inflammatory reaction in the form of redness on the skin due to the reaction of the skin to chemicals such as strong alkalis, strong acids, solvents and detergents (Toding and Zulkarnain, 2015). The onset of erythema in volunteers may be due to a skin condition (Pradana and Nugroho, 2016).

Conclusion

The formulation of frankincense gum extract deodorant spray preparation is more optimal in inhibiting the growth of *Staphylococcus epidermidis* bacteria with an average inhibitory zone of 4-5.35 mm.

Conflict of Interests

The author (s) declares that there is no conflict of interest in this research and manuscript.

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