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PHYTOCHEMICAL SCREENING, FORMULATION AND EVALUATION OF FOOTSPRAY CONTAINING HONEY PINEAPPLE PEEL EXTRACT (*Ananas Comosus* [L.] Merr)

Dwi Endah Kusumawati, Azmi Rahmadani, Rega Putri Puspitasari, Fahreza Maulana Ghazi, Ahmad Nida'ul Husna

Department of Pharmacy, Faculty of Pharmacy, Sultan Agung Islamic University, Semarang 50112, Indonesia

dwiendahkusumawati@unissula.ac.id

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ABSTRACT

Foot odor or bromhidrosis occurs due to the activity of bacteria that convert leucine in sweat into isovaleric acid which results in unpleasant odors. Honey pineapple peel (*Ananas comosus* [L.] Merr) is typically considered waste, but it is known to contain antibacterial compounds. The aim of this study is to explore the potential of honey pineapple peel as an active ingredient in footspray, based on the results of phytochemical screening and physical evaluation. Honey pineapple peel was extracted by maceration method using ethanol 70% then phytochemical test. The preparations were made with various extract concentrations: 0%, 25%, 35% and 45%. These preparations were then tested for organoleptic properties, pH, specific gravity, viscosity, homogeneity, spreadability, and dry time during storage period of 0, 7, 14 and 21 days at room temperature. This study showed that honey pineapple peel extract contained flavonoids, tannins, phenols and alkaloids. The footspray has brown color with honey pineapple scent, pH of 5.71-6.37, specific gravity 1.05-1.09 g/mL, viscosity 7.2-13.2 cP, spreadability 6.67-7.60 cm, and the dry time of 4-5 minutes. Footspray containing honey pineapple peel extract with concentrations of 25%, 35% and 45% has a pH value that is safe for the skin, with good specific gravity, viscosity, homogeneity and spreadability, also stable during storage. Among the tested formulas, formula 1 (25% extract concentration) was preferred due to its soft texture and non-sticky feel when applied to the skin, compared with formula 2 and 3.

Keywords: Evaluation; Extract; Footspray; Honey Pineapple Peel

1. INTRODUCTION

Foot odor is a common problem that can lead to feelings of discomfort, unconfident and may indicate poor personal hygiene (Ashfia et al., 2019). Certain bacteria such as *Staphylococcus epidermidis* and *Bacillus subtilis* contribute to foot odor by breaking down branched-chain of amino acids (leucine or valine) in sweat into isovaleric acid at the plantar skin site. The human foot provides an ideal environment for the colonization of these bacteria due to its high nutrients and humidity (Stevens et al., 2015).

Various methods have been attempted to combat foot odor, including maintaining good hygiene by washing feet with antibacterial soap, drying feet before wearing shoes, soaking feet in salt or lemon solution, using foot powder, and wearing socks and change them frequently. However, these efforts are still ineffective and inefficient. Currently, foot odor control product is available on the market, including: antibacterial soap, foot powder, foot deodorant, foot scrub, while foot odor eliminator spray is remained underdevelop.

Foot spray or foot sanitizer is a foot cleanser that typically contains alcohol as an active ingredient to eliminate microbes or bacteria on the surface of the skin without having to rinse it

with water. Unfortunatelly, the use of alcohol as an antibacterial agent can cause irritanting effects for some people, therefore the alternative way by using natural ingredients (Riyanta et al., 2020). Natural ingredients in foot spray preparations have been extensively studied, including: citronella essential oil (Balfas & Rahmawati, 2022), beluntas leaf (Farhamzah et al., 2021), basil leaf extract (Risnayanti & Dalimunthe, 2022), coffee seed and ginger extract (Amananti & Dairoh, 2021) and betel leaves extract (Iswandana & Sihombing, 2017).

Honey pineapple (*Ananas comosus* [L.] Merr) is a popular fruit that leaves approximately 30% of skin waste after consumption, which contains carbohydrates and various other beneficial secondary metabolites. Honey pineapple peel also exhibit antibacterial activity against *S. epidermidis* and *Streptococcus pyogenes* (Musika et al., 2021), *Bacillus subtilis* and *Streptococcus mutans* (Halima et al., 2020). Wandono et al., 2020 succesfully converted honey pineapple dry skin waste into bioethanol through fermentation and distillation processes. Utilization of pineapple peel ethanol extract in liquid soap formulations is also known to inhibit the growth of *S. aureus* bacteria (Rahmawati et al., 2021). Pineapple peel extract applied to hand sanitizer products is also known to have antibacterial activity against *S. aureus* and *Escherichia coli* (Rini et al., 2017).

Despite the numerous studies on honey pineapple peel, it is often discarded as waste or used as animal feed, indicating its underutilization. However, honey pineapple peel contains bromelain, flavonoids, tannins, alkaloids, phenols, and saponins, all of which possess antibacterial properties (Susanti et al., 2021; Rahmawati et al., 2021; Zharfan et al., 2017). This potential makes honey pineapple peel an inexpensive and readily available active ingredient in anti-odor foot spray preparations. Therefore, this study aims to determine the potential of honey pineapple peel waste as an active ingredient in anti-odor foo tspray focusing on the results of phytochemical screening and assessing its physical properties to ensure safety and efficacy when applied to the skin.

2. METHODS

2.1. Extraction of honey pineapple peel

Honey pineapple peel was obtained from Belik village (Pemalang, West Java). The extract was prepared by maceration method using 70% ethanol as a solvent with a ratio of 1:10. The mixture was left for 6 hours while occasionally stirring then leaving it for 18 hours. The maserate was filtered using filter paper (Filtrate I), then the pulp was re-macerated with 70% ethanol for 2 days then filtered with the same procedure (Filtrate II). Filtrates I and II were evaporated using a rotary evaporator to obtain a thick extract (Rahmawati et al., 2021).

2.2. Phytochemical Screening of Honey Pineapple Peel Extract

2.2.1. Flavonoid Test

A total of 1.0 gram of extract is added to 10 mL of hot aquadest. The mixture is then boiled for 5 minutes, then filtered. After heating, 0.1 g of Mg powder and 1 mL of concentrated HCl were added to the 5 mL of the filtrate. A positive result for flavonoids is when an orange precipitate is formed.

2.2.2. Saponin Test

A total of 0.5 gram of extract was added to 10 ml of hot aquadest in a test tube, then shaken vigorously for 10 seconds. Positive results are indicated by the formation of foam and can last for 10 minutes. A positive result contains saponins if the foam does not disappear after adding 1 drop of 2N HCl.

2.2.3. Tannin Test

The extract was dissolved in warm aquadest and filtered, then dripped with 2 drops of 0.1N FeCl solution. The positive result of tannins is the formation of a blackish brown solution.

2.2.4. Phenolic Test

The extract was put into a test tube, then $FeCl_3$ reagent was added in ethanol solution, positive results were indicated by the formation of green, red purple, blue and black colors.

2.2.5. Alkaloid Test

A total of 0.5 g of extract was added with 1 ml of HCl and 9 ml of aquadest, heated for 2 minutes, cooled and then filtered. As much as 3 drops of the filtrate are added with 2 drops of Mayer's reagent. A positive result if a white or yellow precipitate is produced.

Honey pineapple skin extract is used as an active ingredient in footspray. Each formula uses a ratio of honey pineapple skin extract and 70% ethanol, namely 25%, 35%, 45% and 0%. Footspray is made by mixing the extract with ethanol, then adding glycerin and methyl paraben. After that, the mixture was stirred until homogeneous. Footspray containing honey pineapple peel extract was tested for physical properties including: pH, specific gravity, viscosity, homogeneity, spreadability, and stability during storage.

2.3. Physical Properties of Footspray Containing Honey Pineapple Peel Extract

2.3.1. Organoleptic Test

The odor, color and aroma were observed qualitatively on days 0, 7, 14 and 21 at room temperature.

2.3.2. Test the Degree of Acidity (pH)

pH measurement was carried out using a pH meter. Initially the electrodes were calibrated with standard buffers pH 4 and pH 7. Then the electrodes were dipped into the preparations. The pH value that appears on the screen is recorded. Measurements were made on days 0, 7, 14, and 21 at room temperature.

2.3.3. Specific Gravity Test

The specific gravity test is carried out with a pycnometer which has been dried and weighed. The sample is cooled to 25 °C and put into the pycnometer, tightly closed and then weighed. The sample results were compared with the specific gravity of aquadest.

2.3.4. Viscosity Test

The viscosity was checked using a Brookfield viscometer. A footspray preparation of honey pineapple peel extract is put into a beaker glass. Viscosity measurements were carried out using spindle number 3 until it was submerged and the speed was 100 rpm. Viscosity measurements were repeated 3 times, and carried out on days 0, 7, 14 and 21.

2.3.5. Homogeneity Test

The homogeneity test was carried out by spraying the footspray preparation on a glass object, then leveling it by attaching another glass object, then observing whether or not there were particles that had not been mixed homogeneously. Homogeneity checks were carried out on days 0, 7, 14, and 21.

2.3.6. Spreadability Test

Footspray was sprayed on mica plastic at a distance of 5 cm then the spread was measured using a ruler.

2.3.7. Dry Time Test

The dry time test of the footspray preparation was applied to the inside of the volunteer's arm and then the time was measured using a stopwatch until the preparation was dry.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Screening

Extraction of honey pineapple peel was carried out by maceration using 70% ethanol and then analyzed for phytochemicals. Ethanol as a solvent is based on the ability of ethanol to

dissolve nonpolar, semipolar and polar active ingredients. Ethanol is not toxic and has advantages in extracting secondary metabolites from plants which are antioxidants and antibacterial (Afrina et al., 2018). Based on the results of the phytochemical screening, the ethanol extract of honey pineapple peel contains flavonoids, phenols, tannins and alkaloids (**Table 1**).

This result accordance with research conducted by Rahmawati et al., (2021) and Zharfan et al., (2017), that honey pineapple skin contains bromelain, flavonoids, tannins, alkaloids and phenols which have antibacterial activity. The honey pineapple peel secondary metabolites are the same as regular pineapple peels, namely flavonoids, tannins and saponins (Rini et al., 2017). However, in this study no saponins were detected in the honey pineapple peel ethanol extract. These results are the same as studies conducted by (Namrata et al., 2017) and (Waznah et al., 2021) where the ethanol extract of pineapple peel does not contain saponins. According to (Namrata et al., 2017) the presence of a secondary metabolite compound can be influenced by the type of solvent, extraction method or climatic conditions where the plant grows.

Table 1. Phytochemical screening of honey pineapple peel extract

Secondary Metabolites	Results
Flavonoids	+
Alkaloids	+
Tannins	+
Phenols	+
Saponins	-

Key: (+) = Present; (-) = Absent

3.2. Formulation of Foot Spray Containing Honey Pineapple Peel Extract

Basically, foot spray is the same as hand sanitizer, which is used to kill germs, in this case harmful bacteria. In its formula composition, the foot spray contains ethanol as a solvent, an emollient as a moisturizer so that the surface of the skin of the feet remains soft, and a preservative so that the preparation lasts during the storage period. However, according to (Riyanta et al., 2020) the utilization of alcohol as a solvent has the potential to cause dryness and irritation to the skin, so it is necessary to add other ingredients such as emollients and natural active ingredients that also have antibacterial activity, in this case honey pineapple peel extract. In this research, footspray was made with several combinations of honey pineapple peel extract concentrations, namely 25% (formula 1), 35% (formula 2), 45% (formula 3), and 0% (formula 4, as a control). (**Table 2**).

Table 2. Formulation of footspray containing honey pineapple peel extract

Material	Formula				Function
	I	II	III	IV	
Honey pineapple peel extract	25 g	35 g	45 g	-	Active Subtance
Glycerin	10 %	10%	10%	10%	Emollient
Methyl Paraben	0.2 %	0.2 %	0.2 %	0.2 %	Preservative
Ethanol 70%	Ad 100 mL	Ad 100 mL	Ad 100 mL	Ad 100 mL	Solvent

3.3. Physical Properties of Footspray Containing Honey Pineapple Peel Extract

Organoleptic observations were carried out by observing the color, aroma and texture of the footspray preparations. Honey pineapple peel extract footspray formulations 1, 2, and 3 have the characteristics of a brown color, with a honey pineapple fragrance and a soft texture but tend to be sticky when applied to the skin (**Table 3**). The brown color of footspray comes from honey pineapple peel extract which is brown in color (**Figure 1**). During the storage period of 0,7,14 and 21 days there was no difference in color, aroma and texture of the footspray.

Table 3. Organoleptic test of footspray containing honey pineapple peel extract

Observation	Formula 1	Formula 2	Formula 3	Formula 4
Color	Brown	Brown	Brown	Clear
Aroma	Honey pineapple	Honey pineapple	Honey pineapple	Alcohol
Texture	Soft, slighty sticky on the skin	Soft, sticky to the skin	Soft, sticky to the skin	Soft, not sticky

Figure 1. Footspray containing honey pineapple peel extract

The pH test aims to determine the pH of the footspray preparation so that it is safe when applied to the skin. According to Santoso & Riyanta (2019) the pH of preparations that are safe for the skin is 4-6.5, while according to Riyanta & Febriyanti (2018) the standard pH for topical preparations is 5-7 because it adjusts to the skin's natural pH. Based on the observations, the pH of all footspray preparations of honey pineapple peel extract produced was in the range of 5.71-6.37 (**Table 4**), which means it was suitable and safe for the skin.

Table 4. pH value of footspray containing honey pineapple peel extract

Day-	Formula 1	Formula 2	Formula 3	Formula 4	Standard
0	5.71	5.71	5.76	5.75	**4-6.5**
7	5.93	5.93	5.90	5.98	
14	6.35	6.37	6.37	6.37	
21	5.72	5.72	5.73	5.75	

Another physical evaluation of footspray preparations that is carried out is the specific gravity test. The results of testing the specific gravity of the footspray e preparation are presented in

Figure 2. Based on the results, the specific gravity of formula 1 was 1.05-1.07 g/mL, formula 2 was 1.06-1.09 g/mL, formula 3 was 1.09 g/mL, and formula 4 was 0.94-0.95g/mL. In **Figure 2**, addition of honey pineapple peel extract increased the specific gravity of the preparation. Formula 3 has the highest specific gravity value because it contains more honey pineapple skin extract than formulas 1 and 2. The more concentration of the extract used, the higher the specific gravity value. This is in accordance with Santoso & Riyanta (2019) which states that the concentration of the extract affects the specific gravity of a preparation. During the storage period of 0, 7, 14 and 21 days at room temperature the specific gravity values of the preparations tended to be stable.

Viscosity is a parameter that describes the thickness of a preparation. Based on the result in **Figure 3**, formula 1 has a lower viscosity value than formulas 2 and 3. The viscosity value is directly proportional to the specific gravity of the preparation, Santoso & Riyanta (2019) stated that the smaller the specific gravity value, the lower the viscosity. A low viscosity value describes the more fluid a preparation is and easy to be sprayed or applied to the skin. This means that the addition of honey pineapple peel extract will increase the viscosity value of the preparation. In **Figure 3**, we can see that the viscosity values of all formulas have increased during the storage

period, but they are still classified as liquid and organoleptically do not change the texture of the preparations. The specific gravity tends to stable during the storage, but the viscocity appears to increase due to the interaction between glycerin. Glycerin acts as a humectant which has the ability to bind water components from the air, so that the size of the molecule changes and causes an increasing of the footspray's viscosity.

Homogeneity test aims for the presence of solid particles or lumps that may be present in the footspray which can later affect the spreadability when applied to the skin. Based on the result, all footspray formulas did not show any lumps or solid particles during the storage period of 0.7, 14 and 21 days (**Table 5**).

In the context of application to the skin, the spreadability test is one of the parameters that is measured to find out the spread of the footspray using a spray applicator, where the wide coverage will support the contact between the footspray and the skin, so that the absorption of active substances into the skin is better. The addition of extracts to formulas 1 and 2 had no significant effect on the spreadability, but the more extraxt concentrations used, the less spreadability of the footspray. A dry time test was also carried out to find out how long the footspray can dry when applied to the skin. Based on the results, the dry time of formula 3 is longer than other formulas. The more honey pineapple peel extract used, the more concentrated the footspray preparation will be, so that the dry time of the footspray when applied to the skin is longer.

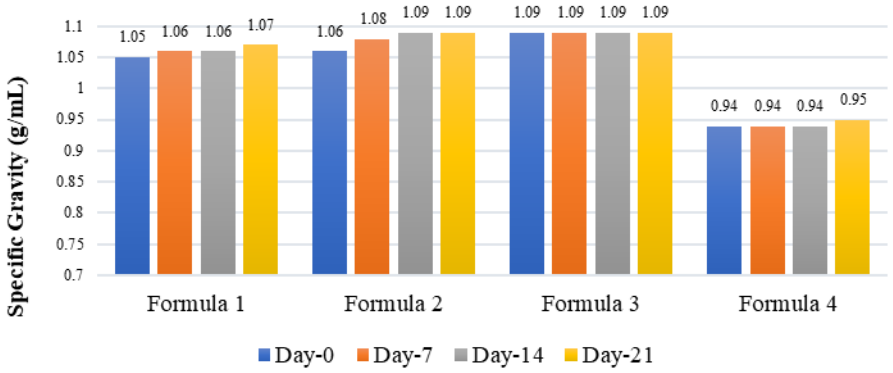

Figure 2. Specific gravity of footspray containing honey pineapple peel extract (g/mL)

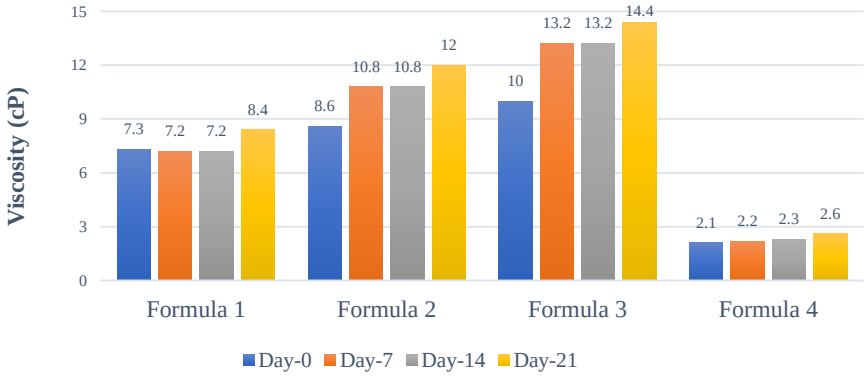

Figure 3. Viscosity value of footspray containing honey pineapple peel extract (cP)

Table 5. Test of homogeneity, spreadability and dry time of footspray containing honey pineapple peel extract

Parameter	Formula 1	Formula 2	Formula 3	Formula 4
Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous
Spreadability	7.23 cm	7.35 cm	6.95 cm	7.61 cm
Dry time	4 minutes, 27 seconds	4 minutes, 38 seconds	5 minutes, 9 seconds	2 minutes, 7 seconds

4. CONCLUSION

Honey pineapple peel extract were positive for flavonoids, tannins, phenols and alkaloids. The ethanol extract of honey pineapple peel can be formulated into an active ingredient with brown color with honey pineapple scent, pH of 5.71-6.37, specific gravity 1.05-1.09 g/mL, viscosity 7.2-13.2 cP, spreadability 6.67-7.60 cm, and the dry time is 4-5 minutes. Formula 1 (25% extract concentration) tends to be preferred because its texture is soft and not too sticky when applied to the skin when compared with formula 2 and 3.

Recommendations for future research include conducting tests to evaluate the antibacterial efficacy of the foot spray containing honey pineapple peel extract against S. epidermidis and B. subtilis and to test the physical stability of the preparations at different storage conditions, namely room temperature (28 ± 2 ºC), high temperature (40 ± 2 ºC), and low temperature (4 ± 2 ºC).

5. CONFLICT OF INTEREST

All authors declare no conflict of interest.

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