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Pandan leaves extract (*Pandanus amaryllifolius Roxb*) as a food preservative

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ABSTRACT

Background: Food poisoning or foodborne disease is still a serious concern in Indonesia. The use of harmful synthetic chemical preservatives are still widely found in society. Therefore, it would require efforts to develop safe natural preservatives— for instance, from Pandan leaves (*Pandanus amayllifolius Roxb*) which are often used as a natural food coloring and flavor concentrates, and has also widely known to have anti-bacterial activities.
Objective: This study aims to determine the potential of pandan leaves extract (*Pandanus amaryllifolius Roxb*) in lowering the total plate count and the number of mold on traditional food.
Methods: After making the water of pandan leaves extract, a phytochemical test was done to determine bioactive compounds, such as tannin, alkaloids, flavonoids, and polyphenols which has anti-microbial properties. In order to determine its food preservatives capabilities, Total Plate Count and Number of Mold was used on a traditional food called Putu Ayu.
Results: Pandan leaves extract contain bioactive compound like tannin, alkaloids, flavonoids, and polyphenols. There were no colonization found in Putu Ayu that was added 15% pandan leaves extract concentration in 101 dilution. The result of Number of Mold Count of Putu Ayu that contain 15% pandan leaves extract concentration showed no mold growth in 104 dilution but failed to provide good fragarance and flavor in food products.
Conclusion: Pandan leaves extract in the concentration of 15% were able to decrease Total Plate Count and Number of Mold in traditional food.

Latar belakang: *Keracunan pangan atau foodborne disease masih menjadi masalah yang serius di Indonesia. Penggunaan pengawet kimia sintesis berbahaya masih ditemukan di masyarakat. Oleh karena itu diperlukan upaya pengembangan pengawet alami yang lebih aman, diantaranya dari tanaman. Pandan wangi, (*Pandanus amayllifolius Roxb*) yang sering dimanfaatkan daunnya sebagai bahan pewarna dan pemberi aroma. telah diteliti memiliki aktivitas antibakteri.*
Tujuan : *Penelitian ini bertujuan untuk mengetahui potensi ekstrak air daun pandan wangi (*Pandanus amaryllifolius Roxb*), dalam menurunkan angka lempeng total dan angka kapang pada pangan tradisional.*
Metode: *Pembuatan ekstrak air daun pandan wangi dan uji fitokimia untuk mengetahuai senyawa bioaktif tanin, alkaloid, flavonoid dan polifenol yang berperan sebagai zat anti mikroba. Untuk pengujian sebagai bahan pengawet pangan adalah Angka Lempeng Total (ALT) dan angka kapang/khamir pada pangan tradisional yaitu putu ayu.*
Hasil:*Ektrak Air Daun Pandan Wangi mengandung senyawa bioaktif tanin, alkaloid, flavonoid,saponin dan polifenol. Tidak ditemukan koloni pada uji angka lempeng total pada ekstrak air daun pandan pada dosis 15% pengenceran 101, dan tidak ditemukan koloni pada uji kapang khamir pada dosis 15% sampai pengenceran 104 namun tidak mampu memberikan aroma dan rasa yang baik pada produk pangan.*

Kesimpulan: *Ekstrak air Daun Pandan Wangi pada konsentrasi 15% mampu menurunkan angka lempeng total dan angka kapang/khamir pangan tradisional.*

INTRODUCTION

Food poisoning and foodborne diseases, especially those that are caused by bacterial pathogens, is still a serious concern in Indonesia and a lot of other places in the world. Based on the data collected by the National Centers of Poisoning Information in Indonesian Food and Drugs Administration (BPOM), throughout 2011, the incidence of food poisoning was reported to be the highest at 58% (601 cases).[1] Based on the parameter of Microbial Contamination test in 4.808 school snacks samples, it was known that : 789 samples (16,41%) exceed the expected Total Plate Count (TPC), 570 samples (11,86%) exceed the allowed Coliform bacterial contamination, 253 samples (5,26%) exceed the allowed Number of Mold (NM), 149 samples (3,10%) were contaminated by Escherichia Coli, 18 samples (0,37%) were contaminated by Staphylococcus Aureus, and 13 samples (0,27%) were contaminated by *Salmonella*.[2]

An effort towards Food Safety is needed to prevent the posibilities of biological and chemical contamination, as well as other objects that can disturb, injure, and harm human health. One of the effort to prevent food containation is often done by using food preservatives, either organics or anorganics compound.[3] Nevertheless, this effort often caused chemical contamination due to the use of forbiden chemical preservatives that are harmful to human bodies. In 2011, the Indonesian FDA (BPOM) tested 20.511 food products, and the result showed that 2.902 samples (14,15%) did not meet the quality and safety regulation, in which : 151 samples contained Formaldehyde, 138 samples contained borax, 3 samples contained yellow methanyl, 1 samples contained auramine, 197 samples contained rhodamine B, and 1.002 samples exceed the regulated microbial contaminations. [1]

The high prefference to use chemical food preservatives need to be counterbalance with an effort to develop safer natural preservatives. Pandan leaves are often use as additives in a lot of food products, mostly as food coloring and fragarance. But besides those usability, pandan leaves also has anti-bacterial properties.[4] Pandan leaves in addition to ethyl acetate extract showed the highest inhibitory potential, with MIC (Minimal Inhibitory Concentration) and MCK (Minimal Killing Concentration) 1,1%b/v and 6,7%b/v against *Staphylococcus aureus*, as well as 0,5%b/v and 4,5%b/v against Escherichia coli.5 These anti-bacterial properties are due to the contents of flavonoids alkaloids, saponins, tannin, and polyphenol. [6,7] The applicative and practical usability for the public, demands a substance that are more eco-friendly, inexpensive, and easily available. Therefore, pandan leaves extract has become one of many options than needed to be developed. This study aims to determine the potential of pandan leaves extract (*Pandanus amaryllifolius Roxb*) in lowering the microbial contamination count, measured by Total Plate Count and the Number of Mold, on food products.

METHODS

Study Design

This study used a post test only with control group design, in which independent variables were the usage of Pandan leaves extract (*Pandanus amaryllifolius Roxb*.) in a traditional food called Putu Ayu, and dependent variable were Total Plate Count and Number of Mold.

Study Period and Location

This study was done in 2014, at the Farmakognosi Laboratory and Microbiology Laboratory of Poltekkes Bhakti Setya Indonesia, Yogyakarta

Pandan Leaves Extract

Pandan leaves (*Pandanus amaryllifolius Roxb*.) were obtained from an area called Gedong Kuning, Yogyakarta. The determination of Pandan leaves plant (*Pandanus amaryllifolius Roxb*.) was done at the Biology Laboratory of Pharmaceutical Faculty of Universitas Gadjah Mada. Determination was done by observing the morphology of the plants based on the refference in "Flora of Java" literature.[8]

Pandan leaves were washed under running water to clean them from dust and other foreign

objects. They were chopped to ease the drying process. Drying was done under indirect sun light, using a black cloth as a cover above the samples. The dry products were, then, crushed using a blender machine, and then sifted using a 40 mesh sieve so that a similarly sized powder was obtained.[9] Then, the dry powder was extracted using Maceration method. Maceration was done by soaking the dry powder in water for 5x24 hours.[10] The obtained extracts were, then, concentrated to remove the excess liquid and determine the random percentage. The concentrated process was done by heating the extract above a waterbath. The concentration obtained from these extraction process using water was 17,96%.

Phytochemical Test
Polyphenol Test

As many as 0.10 gram of the extract was added with 5 ml aquades and boiled for 5 minutes. Then, it was filtered to obtain its filtrate. Filtrate was added with 5 drops of $FeCl_3$ 1%, and color changing was observed. Color changing into bluish green to black showed the prescence of Polyphenol. [11]

Flavonoids Test

As many as 0.10 gram of the extract was mixed with 5 ml ethanol and then shaken, heated, and re-shaken. Next, the filtrate was taken from this mixture. Filtrate was added with 0.20 gram Mg powder and 3 drops of HCl. The formation of red color in the ethanol layer showed the prescence of flavonoids. [11]

Alkaloids Test

As many as 0.50 gram of the extract was added with 5 ml chloroform and 3 drops of amonia. The chloroform fraction was separated and added with 10 drops of H_2SO_4 2M. The acidic layer was separated into 3 parts and was called part A, B and C. Layer A was added with Meyer reactant, layer B was added with Dragendorf reactant, and layer C was added with Wagner reactant. Sediment formation was observed.

The prescence of alkaloids was marked by the formation of white sediment by Meyer reactant, red sediment by Dragendorf reactant, and brown sediment by Wagner reactant.[4]

Tannin Test

As many as 0.50 gram of the extract was added with 5 ml aquades and was boiled for 5 minutes. The mixture was filtered to obtained its filtrate. Filtrate was added with 5 drops of $FeCl_3$ 1%. Color changing was observed. The formation of dark blue or blackish green color showed the precence of tannin.[12]

Saponin Test

As many as 0,5 gram of the extract was put into a test tube that contain 1 ml of aquadest. It was stirred and then added with 1 drops of Hydrochloric acid 2N. The test tube was obserbved to determine the formation of a stable foam.

Saponin test was done by shaking the samples strongly. The formation of, at least, 3 cm stable foam after acid was added, showed the prescence of Saponin. Saponin has a hidrophylic and hydrophobic compound. While being shaked, the hidrophylic compound would bind with water, while the hydrophobic compound would bind with air, hence forming a foam. The addition of acid was necessary to increased the polarity of hidrophylic compound, so that the foam would be more stable. [13]

Putu Ayu

The production of Putu Ayu was done based on a research by Suter 14 which had been modified with the addition of pandan leaves extract. 1 egg was whisked and then mixed into 100 grams of white sugar, 100 grams of all-purpose flour, 100 ml of coconut milk, 50 grams of grated coconut, and pandan leaves extract. The batter was divided into 2 parts, in which one was added pandan leaves extract 15% and the other was added pandan leaves extract 10%. Then, the batter was poured into molds and steamed in medium heat until ready.

Microbes Contamination Test
Total Plate Count Test

First, 1 grams of solid samples were diluted with 10 ml of diluent in a sterile Erlenmeyer flask. Samples were continously diluted until up

to 10 times, 100 times, and 1000 times dilution.

1 ml of each dilution was inserted into sterile Petri dish which had been given sample's number marker containing the level of dilution and the date of examination. Another Petri dish was filled with 1 ml of diluent as a Control Plate to verify the sterility of tools, reagents, rooms, and procedures. After that, into each Petri dish that already contained samples and control, sterile 45 – 500C Media Plate Agar was added as much as 15 - 20 ml.

Bacterial colonization that grew in the Petri dish was counted. Total Plate Count was calculated with the proviso that the number of colony per-plate that may be calculated was between 30 - 300 CFU (colony forming unit). After multiplying with the concentration of the dilution, the number of bacterias in 1 gram/ 1ml of examined sample would be achieved. [15]

Number of Mold Test

Mold contamination in the Putu Ayu samples was done using the method of performing three times replication of the total Number of Mold Test. Sterile PDA medium which had been liquefied and cooled in the temperature of 40°C was added 1ml/L of Chloramphenicol and then poured into Petri dish until frozen. 1 ml of suspension as the result of sample dilution was added into the surface of PDA medium which had been frozen in the Petri dish and containing Chloramphenicol. Then, it was spreaded using spreader glass. Medium and the diluent was used as control. Next, the Petri dish was incubated in the temperature of 20-25°C for 3-5 days. Examination of colonize molds that grew in the medium was performed in accordance with TPC test. [16]

Data Analysis

Data was analyzed using a statistical technique called One-Way Anova test with Confidence Interval 95%.

RESULTS

The determination of Pandan leaves plant (Pandanus amaryllifolius Roxb.) showed that the plant samples were in accordance with the intended. Phytochemical test showed that Pandan leaves extract contained tannin, alkaloids, flavonoids, saponin, and polyphenol (Tabel 1).

Tabel 1. Result from Phytochemical test of Pandan Leaves Extract

Phytochemical Test	Reactant	Result	Conclusion
Alkaloids	Mayer	Sediment formation White	+
	Wagner	Sediment formation Brown	+
	Dragendorf	Sediment formation Red	+
Tannin	FeCl3 1%	Color changing Bluish Green	+
Saponin		Stable Foam formation	+
Flavonoids	Mg+HCL+etanol	Color changing Red	+
Polyphenol	FeCl3 1%	Color changing Bluish green	+

Note: (+) = contain test's compound (-) = not contain test's compound

The result of Total Plate Count with 2 variations of pandan leaves extract concentration showed an inhibitory property in microbial growth, and there were no colonization found in Putu Ayu that was added 15% pandan leaves extract concentration in 101 dilution. Other food products that were given synthetic food coloring showed more bacterial growth (Tabel 2).

Tabel 2. Result of Total Plate Count of Traditional Food after Adding Pandan Leaves Extract

Interventions	Total Bacterial Colony Count :						
	controlmedium	10^1	10^2	10^3	10^4	10^5	10^6
Food without extract (synthetic coloring product)	1	4	20	240	800	800	1600
Food + Pandan leaves extract 10%	1	42	33	96	200	400	3200
Food + Pandan leaves extract 15%	1	0	2	2	9	124	176

The potential anti-fungal effects of pandan leaves was also tested. The result of Number of Mold count can bee seen in Table 3. Putu Ayu that contain 15% pandan leaves extract concentration showed no mold growth in 104 dilution, while samples with 10% concentration showed mold colonization (Table 3).

Tabel 3. Result of Number of Mold count in Traditional Food After Adding Pandan Leaves Extract

Interventions	Total Bacterial Colony Count :						
	controlmedium	10^1	10^2	10^3	10^4	10^5	10^6
Food without extract (synthetic coloring product)	2	0	39	41	19	12	4
Food + Pandan leaves extract 10%	1	0	0	27	14	14	22
Food + Pandan leaves extract 15%	1	0	9	17	0	0	0

Organoleptic observation showed that the addition of pandan leaves extract were able to inhibit mold growth and prevent microbial parasite contamination (flyblow). However, the observation also showed that the addition of pandan leaves extract were not able to give appropriate flavor and fragarance in comparison to the addition of fresh pandan leaves. The food product turned brown instead of green. It also tasted bitter and there was no fragarant aroma. Hence, the pandan leaves extract are currently not applicable to become food preservatives (Tabel 4).

Tabel 4. Result of organoleptic observation in traditional food product that was added pandan leaves extract

Interventions	Organoleptic Observation					
	After 24 hours			5 x 24 hours		
	Color	Aroma	Texture	Color	Aroma	Texture
Food without extract (synthetic coloring product)	Green	Normal fragarant	Normal	Blackish green	Unpleasant odor	Contain maggot
Food + Pandan leaves extract 10%	Brown	No aroma/ bitter	Normal	Brown	No aroma/ bitter	normal
Food + Pandan leaves extract 15%	Dark brown	No aroma/ bitter	Normal	Dark brown	No aroma/ bitter	normal

DISCUSSION

Phytochemical screening was done to determine the chemical compounds of a certain plant. The chemical compound was tested to establish that the plant really contain the intended anti-microbial compound like alkaloids, phenol, flavonoids, carbohydrate, saponin, steroids, and tannin. Acoording to the phytochemical test, pandan leaves extract contain tannin, alkaloids, flavonoids, saponin, and polyphenol.

Tannin is a water-soluble complex polihydrate phenol compound that is toxic to mold, bacteria, and leaven/yeast, as well as inhibits viral growth. Alkaloids are a group of naturally occuring organic nitrogen-containing bases with heterocyclic rings. It has anti-microbial properties due to its ability to impair DNA. Flavonoids has anti-microbial properties due to its ability to merge with bacterial cell membrane and extracellular proteins. Saponin is cytotoxic because it can alter the permeability of cytoplasmic membrane, resulting in the lysis of microbial cells. Polyphenol can be used as an alternative to synthetic antioxidant in the food industry. [17]

The result of TPC test showed that higher concentration of pandan leaves extract in traditional food Putu Ayu resulted in less bacterial colonization. Pandan leaves extract in the concentration of 15% were able to decrease TPC with no microbial contamination in the Putu Ayu samples. However, it has not been measured whether this results are consistent with the Indonesian National Standard (SNI) requirements in which microbial contamination with TPC methods should be less than 106 colony per ml. [18] Pandan leaves extract in the concentration of 10 % still showed microbial growth. This might be affected by the process of drying, chopping, and heating that can alter the amount of extracted compound. The pandan leaves extract was made with water as solvent. Water is polar and non-selective. Eventhough this water extract contain alkaloids, tannin, flavonoids, saponin,and polyphenol, but the concentration of these active chemical compound in the solution might be too little to be able to pierce through microbial cell membrane that tend to be non-polar. [19] This study did not spesifically measure the quantity of those active chemical compounds.

Mold is a type of microorganism that can contaminate food and has the potential to cause poisoning. It has a unique growth pattern which usually grow in cotton-like form in white, black, or other colors. Mold is composed from a lot of cells that combine into one. Leaven/yeast is a non-filament eukaryotic fungi.[21] This study showed that Putu Ayu which contain pandan leaves extract in the concentration of 15% is edible, because it meets the requirement of SNI 7388-2009. SNI 7388-2009 requires the count of Number of Mold that is safe for consumption must be less than 104 colony per ml.[20]

The result of pandan leaves extraction using water as solvent, in this study, was in the concentration of 17,96%. With this method, the determination of water content would affect the refinement of extract. The lesser the water content, then the possibility of fungi contamination would decrease.[21] Voigt (1995) stated that, during extraction process, the amount and types of compounds in the solvent is highly affected by the type of the solvent.[22] According to Cowan (1999) ethanol and methanol is the most commonly used solvent in the extraction of anti-microbial compounds from plants. This is due to their saturated organic and aromatic nature. [23]

Organoleptic observation showed that pandan leaves extract failed to give appetizing flavor and fragarance for Putu Ayu in comparison to the use of fresh pandan leaves. This might be due to the nature of the extract which was in the form of a thicker solution and did not went through freeze drying process. Freeze drying process is better because it can maintain product stability (avoid changing in aroma, colors, and other organoleptic elements), maintain ingredients structure stability (lesser shrinkage and deformation after the drying process), inhibits microbial activities, and prevent chemical or enzymatic reaction that can alter the nutritional properties of a food product. [24] This is coherent with a research by Nawawi et al (2014), in which food products that were added with pandan leaves extract using freeze drying method was

more preferred by consumers, compare to those without freeze drying methods, because there were no changing in flavor, color, and aroma of the food. [25]

CONCLUSION

Pandan leaves extract contain bioactive compound like tannin, alkaloids, flavonoids, and polyphenol. Pandan leaves extract in the concentration of 15% were able to decrease Total Plate Count and Number of Mold in traditional food, but failed to provide appetizing flavor and fragrance.

Development of compound that can give good flavor and fragarance as well as good food preservative potential for food products need to be done.

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