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# PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY TESTS OF TURMERIC TINCTURE AS A DISINFECTANT FOR MEPANDES CEREMONIAL EQUIPMENT IN BALI

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***Abstract***

***Background****: In the traditional Mepandes ceremony of Bali, one of the key practices involves the use of sangging equipment, including chisels, files, and sharpeners, to smoothen teeth surfaces. Typically, one set of sangging tools is shared among 3-10 individuals, rotating their use. This practice poses a potential risk of disease transmission. The current cleaning methods vary, with some using water and turmeric while others soak the equipment in 5% NaOCl. However, the use of water alone may not ensure proper hygiene, and NaOCl usage presents challenges such as equipment odor and potential health hazards.*

***Aims****: The objective of this study is to qualitatively analyze the phytochemical content, inhibitory activity, and total plate count in turmeric tinctures at concentrations of 5%, 10%, and 15%*

***Methods****: The tincture is prepared by soaking turmeric simplicia in 70% alcohol for 5 days at concentrations of 5%, 10%, and 15%. In this study, the negative control is 70% alcohol, the positive control is 5% NaOCl, and the treatment includes tinctures at 5%, 10%, and 15%. Phytochemical tests include flavonoid, tannin, saponin, terpenoid, and alkaloid assays. Antimicrobial activity is assessed through total plate count (TPC) and inhibition tests.* ***The results*** *The research results indicate the presence of tannin, flavonoids, and phenols in all three treatments. Based on the antibacterial tests and total plate count, all three tincture solutions can be used as disinfectants. However, for efficiency, a 5% concentration is recommended as it exhibits the same antibacterial strength as the 10% and 15% tinctures.****Conclusions:****The use of turmeric tincture treatment can be employed as a disinfectant for ceremonial tools since it can inhibit the growth of S. aureus bacteria, and its total plate count value matches that of the positive control.*

***Keywords****: turmeric tincture, Mepandes, antimicrobial*

## 1. Introduction

Mepandes is a crucial stage of the tooth-cutting ceremony known as the 'metatah' ceremony, which is mandatory for Hindu devotees. There are specific treatments for individuals undergoing tooth cutting, and one of these treatments involves the use of sangging tools. In the Mepandes process in Bali, sangging equipment is utilized, which includes

chisels, files, and sharpeners. Sangging tools are employed to smoothen teeth surfaces during the tooth-cutting or Mepandes procedure. Typically, one set of sangging tools serves 3-10 individuals, with the equipment being used in a rotational manner (1). This undoubtedly poses the potential risk of transmitting diseases such as COVID-19, Hepatitis B (HBV), HIV and AIDS, Herpes Simplex, Tuberculosis (TB), Syphilis, Gonorrhea, and Typhoid.

Based on observations, historically, sangging equipment has been cleaned using water, applied with turmeric, and sometimes soaked in 5% NaOCl (sodium hypochlorite). Washing with water alone has proven insufficient in ensuring the hygiene of sangging tools. The use of NaOCl presents challenges, such as equipment odor, and potential irritation and health risks. Disinfectants containing chlorine can be harmful to mucous membranes like the eyes and mouth, making it inadvisable to expose chlorine-containing compounds to parts of the body. Although hypochlorite ions rapidly degrade before being absorbed by living organisms, there are concerns that the accumulation of hypochlorite compounds may lead to the formation of carcinogenic substances (cancer-causing).

Prolonged contact with sodium hypochlorite and other hypochlorite compounds (such as calcium hypochlorite, used in swimming pool disinfection) is not recommended to prevent unforeseen and hazardous consequences. In light of these considerations, there is a need for an alternative method as a safe antimicrobial solution for sangging tools that is culturally compatible and does not pose risks (2).

This research aims to assess the potential of turmeric tincture as a disinfectant for sangging tools. The primary bioactive component in turmeric is curcumin. Studies have demonstrated that curcumin possesses strong antioxidant, wound-healing, and anti-inflammatory properties. Turmeric is a medicinal plant commonly used in traditional medicine and contains antioxidants like polyphenols, tannins, and ascorbic acid. The most dominant antioxidant compound is curcumin, known for its wound-healing and anti-inflammatory properties, especially in acne treatment. Turmeric also has the ability to inhibit the growth of bacteria, viruses in vitro, and fungi (3). Research findings have shown that the best treatment involves the addition of 3% starch concentration and 1% turmeric filtrate (4). Previous studies have indicated that turmeric contains anti-inflammatory substances that can expedite the healing process of ulcers (5). Tincture is a solution containing ethanol and hydroalcohol. The use of alcohol in tinctures facilitates the solubility of active components in turmeric and imparts antimicrobial capabilities.

## 2. Research Material and Methods

### a. Material

The tools used in this study were knives, measuring pipettes (Iwaki) 1 ml, measuring pipettes (Iwaki) 10 ml, ball pipettes, drip pipettes, measuring cups (Iwaki) 500 ml, test tubes (Pyrex), test tube racks, beaker glass (Pyrex) 50 ml, beaker glass (Pyrex) 500 ml, measuring flask (Pyrex Iwaki) 10 ml, measuring flask (Pyrex Iwaki) 100 ml, glass bottles, spatula, stirring rod, porcelain dish, vacuum (Roker 300), vortex (WINA Type 701), micropipette 20µl-1000µl (secorex), yellow tip, blue tip, blender (Philips), analytical balance (radwag), scale, oven, Uv-Vis spectrophotometer (Biochrom), cuvette, electric stove, jar, fume hood.

The main ingredient used in this study is turmeric obtained from the market by random sampling. The materials used for analysis include hydrochloric acid (HCl) (Merck, Germany), methanol PA 96% (Merck, Germany), sulfuric acid (Merck, Germany), 1,1-diphenyl-2-picrylhydrazil (dpph) (SIGMA), quercetin (SIGMA), tannic acid (SIGMA), gallic acid (SIGMA), aquadest, AlCl3 (Merck, Germany), NaNO2 (Merck, Germany) Folin-Denis reagent (Merck, Germany), Na2CO3 (EMSURE),® Folin–Ciocalteau reagent, mayer reagent (Merck, Germany), magnesium powder (Merck, Germany), FeCl3 (Merck, Germany), PCA media (Oxoid, UK), and NaCl diluent solution (Oxoid, UK).

### b. Method

The research stages conducted in this study involve the collection of turmeric rhizomes, drying and grinding them, preparing alcohol for tincture production, phytochemical testing, creating various tincture concentrations, bacterial inhibition tests, and total plate count tests. The preparation of simplicia is carried out, starting from the preparation of turmeric, its drying, and powder production. Tincture is made by soaking turmeric simplicia in 70% alcohol for 5 days at concentrations of 5%, 10%, and 15%. In this research, the negative control is 70% alcohol, the positive control is 5% NaOCl, and the treatment includes tinctures at 5%, 10%, and 15%.

Method The phytochemical test phase includes alkaloid, phenolic, flavonoid, tannin, saponin, steroid, and terpenoid tests.

(a) Alkaloid tests were carried out samples of spice extract as much as 1 mL added 3 drops of H2SO4 2N. Test tube I was tested with meyer reagent, and test tube II with dragendorff reagent. Positive tests of alkaloid compounds are characterized by the presence of orange deposits on Meyer's reagents, and white deposits on Drendorff's reagents (Harborne, 1996).

(b) The phenolic test was performed with the addition of 10 drops of 1% FeCl on 1 mL of spice extract sample. The formation of green, purple, blue, red or solid black

indicates the presence of phenolic compounds (Harborne, 1996).

(c) The flavonoid test was carried out with 1 mL of spice extract sample sample then heated in a bath. The filtrate obtained is added with 0.1 grams of Mg and 2 drops of HCl 6N. The formation of red color indicates the presence of flavonoid compounds.

(d) Tannin Test A sample of 1 mL of spice extract was added with 2-3 drops of 1% FeCl3 solution, and observed the color change that occurred. If it is blue or green, it indicates the presence of tannins. Blue color indicates the presence of 3 hydroxyl clusters in the tannin aromatic core. Green color indicates the presence of 2 hydroxyl clusters in the tannin aromatic core (Ministry of Health of the Republic of Indonesia, 1995)

(e) Saponin test is carried out as much as 1 mL of sample is boiled in 10 mL of water using a water bath, then shaken and let stand for a while. The formation of a stable foam indicates the presence of saponins (Harborne, 1996).

(f) Steroid testing is done by taking 2 mL of each sample then added with 3 drops of concentrated HCl and 1 drop of concentrated H2SO4. If each solution is formed green color then positively contains steroids (Septianingsih, 2013).

(g) Terpenoid tests carried out samples of 2 mL added with 3 drops of concentrated HCl and 1 drop of concentrated H2SO4. If each solution is formed red or purple color then positively contains terpenoids (Septianingsih, 2013).

(1) Flavonoid levels (Singh et al., 2012) Determination of total flavonoids using a spectrophotometer using the AlCl3 method. The extract sample weighed 0.01 g diluted into 10 ml of aquadest. A total of 1 ml of sample was added with 4 ml of aqueous and 0.3 ml of NaNO2 solution (10%). After that, it was incubated for 5 minutes and added 0.3 ml of AlCl3 solution (10%), then immediately tested with a spectrophotometer at a wavelength of 435 nm.

(2) Tannin levels (Suhardi, 1997) The determination of total tannin extracts was analyzed using the Folin-Denis method. A total of 0.01 g of extract is diluted into 10 ml of aquadest. The sample that has been diluted in the pipette as much as 1 ml then added 0.5 ml Folin-Denis reagent, then divortex and added 8.5 ml Na2CO3 10%. The solution is vortex and then incubated for 30 minutes. Absorbance is measured using a spectrophotometer at a wavelength of 725 nm.

(3) Total Phenol Content (Sakanaka et al. 2005) A total of 0.01 grams of extract samples were added aquadest until they reached the limit mark of 10 ml in the measuring flask. The test

sample was pipettes of 0.4 ml placed in a test tube, added 0.4 ml of Folin–Ciocalteu reagent, divortex until homogeneous and allowed to stand 5 minutes before adding 4.2 ml of 10% sodium carbonate solution (NA2CO3). The samples were incubated for 30 minutes at room temperature before reading their color absorption at a wavelength of 760 nm.

(4) Antibacterial Test

The bacterial suspension is pipetted as much as 100µl poured onto Mueller-Hinton media so that it is then flattened using a sterile cotton swab. After that, let stand for 15 minutes. Empty discs are immersed for 15 minutes, then evaporated over aluminum foil. The disc containing the sample is placed on the surface to hygienically fit the laminar air flow. The media is incubated in an incubator at a temperature of 37ºC for 24 hours. The clear zone formed around the disc is measured in diameter using a caliper.

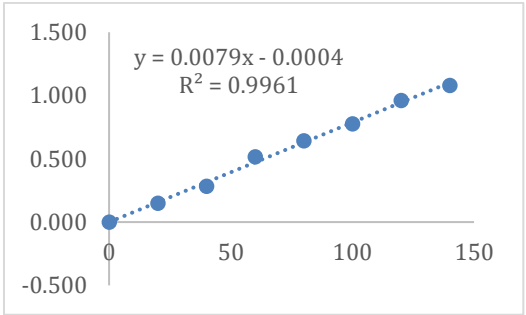
## 3. Results and Discussions

This research has been carried out, generating data such as the results of phytochemical tests, antibacterial tests, and total plate count tests, as presented in Table 1, Table 2, Table 3 and Table 4.

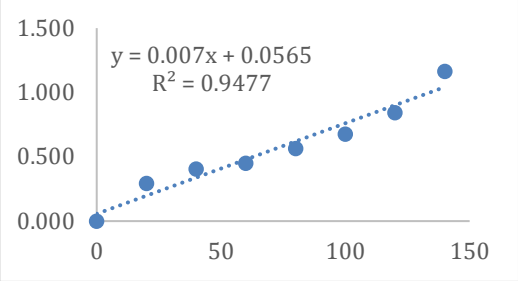
Table 1 indicates the similarity in content across each treatment, such as the presence of flavonoids, phenols, and tannins. Plants produce flavonoids as a defense response to microbial infections, and these substances have demonstrated significant antimicrobial properties against a broad spectrum of pathogenic microorganisms in laboratory settings. The antimicrobial effects of flavonoids stem from their diverse biological activities, which may not initially appear highly specific(6). Flavonoids exhibit a bacteriostatic effect against all the examined bacteria and fungi (7).

Table 1. Phytochemical Test Results for Each Treatment

| No | Test Parameter | Results for Concentration | | |
|---|---|---|---|---|
| | | 5% | 10% | 10% |
| 1 | Alkaloids | Negative | Negative | Negative |
| 2 | Flavonoids | Positive | Positive | Positive |
| 3 | Tannins | Positive | Positive | Positive |
| 4 | Phenols | Positive | Positive | Positive |
| 5 | Steroids | Negative | Negative | Negative |
| 6 | Terpeneoids | Negative | Negative | Negative |
| 7 | Saponins | Negative | Negative | Negative |



Standar Quersetin




Standar Gallid Acid

Various halogenated chalcones, flavanones, and flavones were examined for their antibacterial and antifungal properties in a laboratory setting. The majority of these compounds demonstrated the ability to hinder the growth of bacteria and fungi, especially at lower concentrations. Chalcones, in particular, displayed significant antimicrobial effectiveness against Gram-positive bacteria, notably the S. aureus species(8). The suggested antibacterial mechanisms of flavonoids encompass the following: suppression of nucleic acid synthesis, disruption of cytoplasmic membrane functions, inhibition of energy metabolism, hindrance of attachment and biofilm formation, inhibition of cell membrane porins, modification of membrane permeability, and reduction of pathogenicity (9).

Tannins represent a significant class of compounds in plants that offer potential health advantages. These tannins have been identified as effective in suppressing bacterial growth through a variety of mechanisms, including iron binding, hindrance of cell wall formation, disruption of cell membranes, and inhibition of fatty acid synthesis pathways. Tannins can also function as quorum sensing inhibitors, reducing the expression of numerous virulence factors like biofilms, enzymes,

Table 2. The Results of Flavonoids and Phenol Content in Each Treatment

| No | Code sample | Flavonoids Test (mg/100gQE) | Phenol Test (mg/100gGAE) |
|---|---|---|---|
| 1 | Treatment 5% | 523.93±37.59 | 10857.96±181.01 |
| 2 | Treatment 10% | 906.90±28.90 | 12300.72±46.40 |
| 3 | Treatment 15% | 955.88±71.78 | 11586.65±413.12 |

Description: n = 3

Table 3. Results of Total Plate Count Testing

| No | Code sample | Microba total | |
|---|---|---|---|
| | | before | after |
| 1 | Water | $1{,}1 \times 10^6$ | $2{,}5 \times 10^4$ |
| 2 | Alcohol | $5{,}1 \times 10^4$ | <30 |
| 3 | Byclean | $2{,}5 \times 10^4$ | <30 |
| 4 | Treatment 5% | $1{,}5 \times 10^5$ | <30 |
| 5 | Treatment 10% | $1{,}4 \times 10^5$ | <30 |
| 6 | Treatment 15% | $7{,}8 \times 10^4$ | <30 |

adhesins, motility, and toxins. Furthermore, tannin-loaded nanoparticles/hydrogels exhibit notable antibacterial properties(10). Tannic acid is regarded as a valuable natural substance suitable for eradicating pathogenic agents, and this review encompasses an examination of recent investigations into tannic acid when combined with biopolymers, exploring the active characteristics of the resulting complexes(11).

Based on Table 2 and Table 3, variations in content are evident in each treatment. This variance depends on the quantity of turmeric simplicia used in tincture preparation. According to the results of antibacterial testing and total plate count (Table 3 and Table 4), all three types of tincture solutions can be employed as disinfectants. However, for efficiency, a 5% concentration is recommended, as it exhibits the same antibacterial efficacy as the 10% and 15% tinctures. All treatments exhibit similar activity to other types of disinfectants such as alcohol and Byclean. This indicates that turmeric tincture possesses the ability to inhibit bacterial growth and can be used as a disinfectant for sangging tools during Mepandes ceremonies

Table 4. The results of antibacterial testing against S.aureus

| No | Code sample | The results of antibacterial |
|---|---|---|
| 1 | Treatment 5% | 7.19±0.35 |
| 2 | Treatment 10% | 7.40±0.29 |
| 3 | Treatment 15% | 7.82±0.25 |
| 4 | Chloramphenocol 25 mg | 18.89±2.79 |
| 5 | Etanol 96% | 0±0 |

Description: n = 3

## 4. Conclusions

The research results indicate the presence of tannins, flavonoids, and phenols in all three treatments. Based on the antibacterial testing and total plate count results, all three tincture solutions can be used as disinfectants. However, for efficiency, a 5% concentration is recommended, as it exhibits the same antibacterial strength as the 10% and 15% tinctures.

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