

Identification of Phytochemical Compounds and Antioxidant Activity in Methanol Extract of Basa Genep Seasoning

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ABSTRACT

Basa genep is a basic spice in traditional Balinese cuisine used in making traditional food. The purpose of this study was to identify the densest type, content, and antioxidant activity of methanol extract of *genep bases* using an extraction method whose testing was carried out with a Uv-Vis spectrophotometer (*Biochrom*). The results found that base *genep methanol extract* had flavonoid levels of 1427.90 ± 60.46 mg QE/100g, tannin levels of 3277.05 ± 65.33 mg TAE/100g, total phenol levels of 2398.08 ± 72.195 mg GAE/100g with IC₅₀ values of 144.43 ± 6.673 ppm including medium category

INTRODUCTION

Food found in each region in Indonesia has its own taste, just like traditional food found in Bali. Traditional food for Balinese people is one of the cultures passed down from previous generations. Traditional Balinese food as a culinary tour (*culinary tourism*) which is defined as food that is processed using a combination of food ingredients and spices (*base*) that have a specific taste, aroma, color, and texture. In Bali there are various types of spices used in traditional cuisine such as: *Bumbu Intuk, Bumbu Ulig, Bumbu Rajang and Bumbu Basa Genep* (Widhiantara *et al.*, 2012). Seasonings commonly used in traditional Balinese food processing are seasonings with complete ingredients or commonly referred to as *Genep base*.

Basa genep is a traditional Balinese spice used in making traditional food (Pramana, 2015). Making *genep bases* uses ingredients consisting of herbs and spices. Spice is a plant that is commonly used as a flavoring ingredient as a mixture of dishes or a mixture of basic ingredients. Spices have a more subtle and delicate aroma compared to spices. The seasoning consists of garlic, onion, ginger, lemongrass, and chili. Spices are plants that are used as flavoring ingredients in dishes consisting of ingredients such as cloves, cardamom, cumin, and cinnamon. Spices have a sharper and stronger aroma compared to spices and also tend to have a dry texture, so they can be stored longer without even processing (Broe, 2021).

Spices contain bioactive compounds such as flavonoids, phenols, terpenoids, polyphenols, curcumin, eugenol, vitamins A, C and E and other bioactive substances that act as antioxidants and antimicrobials (Indraguna, 2009). The content of bioactive compounds found in spices has properties as antioxidants that can prevent or delay some types of cell damage due to oxidation processes by oxidants or free radicals (Huang, 2018). This certainly helps in reducing food wastage and allows food to be stored longer, thus *Genep basac* can be developed into functional food. The presence of bioactive compounds contained in spices *Base Genep* It is still not widely known by the public. Therefore, this study conducted research on identification to determine the type, content, and antioxidant activity contained in methanol extract *Genep base*. Identification of bioactive compounds provides a source of information to the public so that it can maximize its application.

LITERATURE REVIEW

Genep Seasoning

Balinese cuisine has its own characteristics in terms of strong taste and aroma. Balinese cuisine is usually rich in the use of spices in the processing of any traditional food. Seasoning in Balinese society is usually referred to as alkaline. *Genep seasoning* or other designations *Genep basais* one of the traditional Balinese spices that has a distinctive taste. *Genep bases* is a special spice found in Bali by using ingredients such as onions, chili, pepper, coriander, kencur, turmeric, galangal, ginger, salt and shrimp paste, so as to make it different from other culinary. The distinctive taste of Balinese cuisine is what is able to attract tourists and culinary connoisseurs and make it a different attraction. Various

kinds of Balinese culinary specialties that usually always use *Genep basa* Such as, betutu, lawar, and be guling (Goddess *et al.*, 2021).

Phytochemical Compounds

a. Flavonoids

Flavonoids represent the largest class of phenolic compounds in nature due to the presence of multiple types of hydroxylation, alkoxylation, and glycosylation levels in the flavonoid structure. Flavonoids have a basic carbon skeleton of 15 carbon atoms forming a C₆-C₃-C₆ arrangement (Figure 1). Flavonoids are polyphenolic compounds that are widely present in plants in the form of glycosides and are combined with sugars. Since flavonoids are polar compounds, polar solvents commonly used to extract flavonoids include methanol, acetone, ethanol, water, and isopropyl alcohol (Syriac *et al.*, 2015). Flavonoids act as antioxidants and are phenolic compounds that are polar and soluble in water.

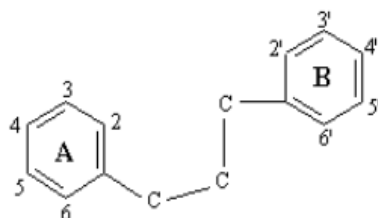


Figure 1. Flavonoid Structure
(Source: Julianto, 2019)

Flavonoids derived from more than 2000 plants have been identified including anthocyanin compounds, flavonols, and flavones. Anthocyanins are pigments that have color, usually found in flowers that have red, purple, and blue colors. This pigment is also found in various other plant parts, such as fruits, stems, leaves and even roots. Flavonoids are mostly collected in the vacuoles of plant cells although the site of synthesis is outside the vacuole. Flavonoids can be grouped based on their structure including chalcones, flavones, flavonols, flavanones, anthocyanins and isoflavones (Julianto, 2019). Flavonoids have antioxidant activity by capturing free radicals and reducing the formation of singlet oxygen (O⁻) (Trianggadewi, 2010). The structure of flavonoids consists of multiple phenolic groups (-OH and aromatic groups) and has conjugated double bonds, so it can resist free radicals. The mechanism of action of flavonoids as antioxidants is direct or indirect. The direct mechanism of flavonoids is to neutralize the toxic effects of free radicals by releasing hydrogen ions, while the indirect mechanism is to increase the expression of endogenous antioxidant genes through various mechanisms (Sumardika and Jawi 2011). The mechanism of increased antioxidant gene expression is based on the activation of nuclear factor erythroid 2-related factor 2, which plays a role in the synthesis of endogenous antioxidant enzymes such as the SOD gene (Sumardika and Jawi 2011).

b. Tannins

Tannins are phenolic compounds that form bitter and astringent/chelates that can react with proteins or other organic compounds and agglomerate. Tannins are also polyphenolic compounds with very large molecular weights, exceeding 1000 g/mol, and can form complex compounds with proteins. Tannins have molecular weights ranging from 500 to 3000 (gallates) and over 20,000 (proanthocyanidins) (Julianto, 2019). The structure of tannin compounds consists of a benzene ring (C6) bonded to a hydroxyl group (-OH) (Figure 2). Tannins play important biological roles due to their functions as protein deposits and metallic agents. Tannin compounds are considered biological antioxidants and play an important role in protecting plants from herbivores and pest predation, as well as acting as regulators of plant metabolism. Tannin compounds have OH groups whose hydrogen atoms can be donated to free radicals to become non-radical compounds (DPPH-H) (Hasan et al., 2022).

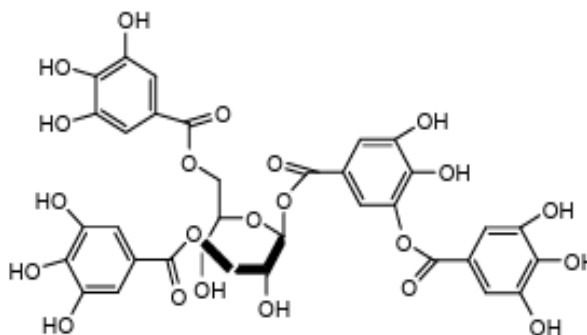


Figure 2. Tannin structure
(Source: Noer *et al.*, 2018)

The chemical properties of tannins generally have phenol groups and are colloidal, in water tannin compounds are colloidal and weak acids. All types of tannins can dissolve in water, with their large solubility will increase when dissolved in hot water. In addition, tannins are also soluble in organic solvents such as methanol, ethanol, acetone and other organic solvents. The physical properties of tannins generally have a high molecular weight and tend to be easily oxidized into a polymer. Most tannins are amorphous and have no melting point. Tannins are yellowish-white to light brown, depending on the source of the tannins (Nofita and Dewangga, 2022). Tannin compounds work as secondary antioxidants with mechanisms of action such as stopping the formation of free radicals by chelating iron metals (Fithriani, 2015). Tannins can suppress the process of lipid peroxidation so as to prevent hypercholesterolemia (Noer *et al.*, 2018).

c. Phenol

Phenolic compounds are secondary metabolite compounds found in plants with the characteristic of having an aromatic ring containing one or two hydroxy groups (OH) (Figure 3). Phenolic compounds are divided into two types of compounds, namely phenolic compounds derived from the mevalonic acetic acid pathway and the sikimic acid pathway. The group of phenolic compounds derived from the acetic acid mevalonic acid pathway is polyketide compounds

and phenolic compounds derived from the acetic acid pathway are phenyl propanoids. Phenolic compounds derived from the combination of these two biosynthetic pathways are flavonoid compounds (Julianto, 2019). Phenol compounds have other names such as carbolic acid, monohydroxybenzene phenate, phenic acid, phenylic acid, phenyl hydroxide, oxybenzene, benzenol, monophenol, phenyl hydrate, phenylic alcohol, and phenol alcohol. Phenol is obtained as a result of coal oxidation (Suminar, 1999).

Phenol is a crystalline substance that is colorless and has a distinctive aroma. Phenol compounds can undergo oxidation so that they act as reducing agents (Hoffmann *et al.*, 1995). Phenols are more acidic than alcohols, but more basic than carbonic acid because they can remove H⁺ ions from their hydroxyl groups. The ion production makes the phenoxide anion C₆H₅O⁻ which can be dissolved in water. Phenol has a melting point of 41 °C and boiling point 181 °C. Phenol has a limited solubility in water of 8.3 g / 100 mL (Fessenden, 1992). Phenol is one of the compounds that can be toxic and corrosive to the skin (irritation) to cause human health problems and death in organisms at certain concentrations. The level of toxicity of phenol varies depending on the number of atoms or molecules attached to the benzene chain (Qadeer and Rehan, 2002).

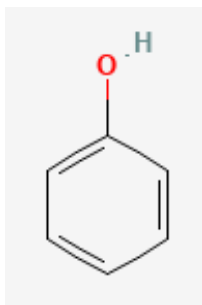


Figure 3. Phenol Structure

(Source: National Center for Biotechnology Information, 2023)

Phenolic compounds are the largest group of compounds that act as natural antioxidants in plants. Phenolic compounds have one (phenol) or multiple (polyphenol) phenolic rings, which are hydroxyl groups attached to an aromatic ring, making them susceptible to oxidation by donating hydrogen atoms to free radicals. The ability to form stable phenoxy radicals during oxidation reactions gives phenolic compounds great potential as antioxidants. Natural phenolic compounds that usually exist in the form of polyphenols, forming ethers, esters or glycoside compounds, including flavonoids, tannins, tocopherols, coumarins, lignin, cinnamic acid derivatives and polyfunctional organic acids (Dhurhania and Novianto, 2019).

Antioxidant

Antioxidant compounds are compounds that can provide electrons (donor electrons). Antioxidants are compounds that can counteract or dampen the negative impact of oxidants that have a certain amount of chemical components so as to inhibit or slow down damage due to the oxidation process (Sayuti and Yenrina, 2015). Antioxidant compounds can also be inhibitors used in inhibiting autooxidation. Antioxidants function by donating electrons to oxidant

compounds, thus inhibiting the activity of these oxidants (Winarti, 2010). The body needs antioxidants to protect it from free radicals. The antioxidant effect of phenolic compounds is based on oxidative properties, which play a role in neutralizing free radicals (Panovska et al., 2005).

Antioxidants are compounds that occur naturally in food. Vegetables and fruits are one of the important sources of antioxidants, and it has been proven that people who consume a lot of vegetables and fruits have a lower risk of suffering from chronic diseases compared to those who consume less vegetables and fruits. This compound serves to protect food from damage caused by oxidation reactions of fats or oils so that food tastes and smells rancid. In the food industry, antioxidants can be used to prevent oxidation processes that can cause damage, such as rancidity, discoloration and aroma, and other physical damage (End *et al.*, 2007). Factors that affect antioxidant activity include lipid content, antioxidant concentration, temperature, oxygen pressure, and chemical components of food in general such as protein and water (Sayuti and Yenrina, 2015).

The main function of antioxidants is to minimize the occurrence of oxidative processes in food and in the body. Antioxidants are expected to inhibit the oxidation of fats and oils, minimize the occurrence of food spoilage processes, extend the life of the food industry, increase the stability of fats in foods, and prevent the loss of sensory and nutritional quality. Lipid peroxidation is one factor that plays a role in damage during food storage and processing (Hernani and Raharjo, 2005). Antioxidants are not only used in the pharmaceutical industry, but antioxidants are also widely used in the food industry, petroleum industry, rubber industry and so on (Tahir *et al.*, 2003). Antioxidants are also able to inhibit the oxidation process which can lead to various degenerative diseases and premature aging.

Compounds that belong to the type of primary antioxidants are Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), catalase and metal-binding proteins. Superoxide dismutase (SOD), GPx, is also known as an enzyme antioxidant. This is an endogenous antioxidant that protects tissues from oxidative damage caused by free radicals such as superoxide anion (O_2^-), hydroxyl radical (OH^-), and hydrogen peroxide (H_2O_2). Some secondary antioxidant compounds include vitamin E, vitamin C, β -caroten, isoflavones, bilirubin and albumin. This antioxidant potential is by cutting the chain oxidation reaction of free radicals or by capturing them (scavenger free radical) so that free radicals do not act with cellular components (Sayuti and Yenrina, 2015). The smaller the IC value indicates that the stronger and greater the ability of the sample as an antioxidant in fighting free radicals according to table 1

Table 1. IC50 Value and Antioxidant Category

IC50 value (ppm)	Antioxidant Category
> 200	Very weak
151-200	Weak
100-150	Keep
50-100	Strong
< 50	Very Powerful

METHODOLOGY

Time and Place of Research

This research will be carried out from May - October 2023 at the Laboratory of Basic Sciences and Food Analysis, Faculty of Agriculture, Warmadewa University.

Tools and Materials

a. Tool

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 round bottom flask (*buchi*), *glass bottle*, *spatula*, *stirring rod*, *porcelain dish*, *vacuum* (Roker 300), *vortex* (WINA Type 701), *micropipette* 20 μ l-1000 μ l (*secorex*), *yellow tip*, *blue tip*, *blender* (Philips), *analytical balance* (*radwag*), *scale*, *oven*, *evaporator* (*buchi*), *Uv-Vis spectrophotometer* (*Biochrom*), *cuvettes*, *electric stoves*, *jars*, *fume hoods*

b. Material

The ingredients used in this study were ginger, galanguh, kencur, turmeric, onion, garlic, cayenne pepper, lombok chili, bangle, jangu, white pepper, black pepper, coriander, nutmeg, candlenut, tabia bun, cloves, mesui, kaffir lime leaves, bay leaves, kaffir bark, p.a methanol solvent (MERCK), pH-indicator strips (*McolorpHastTM*) *1,1-diphenyl-2-picrylhydrazil* (dpph), aquadest, AlCl₃ 10%, NaNO₂ 10%, Folin-Denis reagent, Na₂CO₃ 10% (EMSURE),[®] Folin-Ciocalteau reagent, *mayer reagent*, HCl 6 N, concentrated HCl, H₂SO₄ 2N, concentrated H₂SO₄, magnesium powder, FeCl₃ 1%

Implementation of Activities

This research is a type of research that is descriptive, qualitative and quantitative. Qualitative descriptive research is to determine the types of compounds contained in methanol extract of *genep basaspices*. While quantitative descriptive research to determine the content of phytochemical compounds such as flavonoids, tannins, total phenols and antioxidant activity of IC50. This research was conducted by making a thick extract of *genep basaspices*. This study also made a thick extract of *genep basaspices* which will later be used for comparison on each test parameter. These materials are macerated using methanol solvent for 2 x 24 hours and remaceration is carried out. Thick extracts

will be tested for flavonoid levels, tannin tests, phenol levels and antioxidant activity using the DPPH method (*1,1-diphenyl-2-picrylhydrazil*) with 3 repeats.

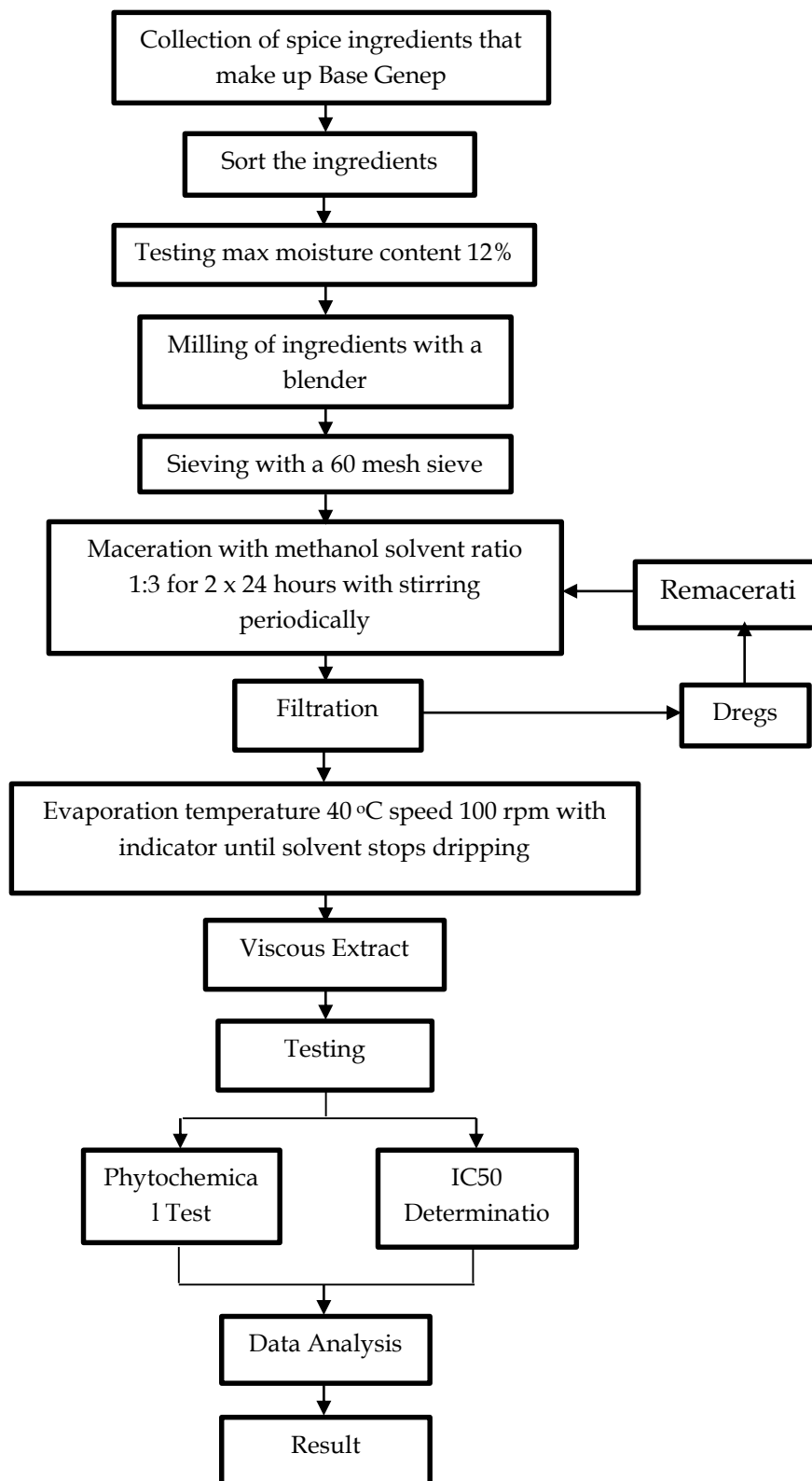


Figure 4. Research Flow Diagram of Identification of Phytochemical Compounds and Antioxidant Activity in Methanol Extract of Spices Constituent of *Genep Basaseasoning*

Data Analysis

This study describes the identification of phytochemical constituents and antioxidant activity in the methanol extract of Genep Basa spice using descriptive data analysis methods.

RESULTS AND DISCUSSION

This study tested phytochemical compounds quantitatively consisting of flavonoid compound levels, tannin compound levels and total phenol compound levels. Quantitative testing aims to determine the total levels of secondary metabolites contained in methanol extract of genep basaseasonings. In addition, antioxidant testing was also carried out on methanol extract of *base genep* spices. Antioxidants are found naturally in food and are used especially in the food industry to prevent damage such as rancidity, discoloration and aroma, and other physical damage (Tamat *et al.*, 2007). The results of the study can be seen in Table 2.

Table 2. Test Results of Phytochemical and Antioxidant Levels
on Base Genep *Methanol Extract*

No	Test Parameters	Base Genep <i>Test Levels</i>	IC50 Category
1	QE Flavonoid Levels (mg/100g)	1427.90±60.46	-
2	Tannin content TAE (mg/100g)	3277.05±65.33	-
3	GAE Total Phenol Content (mg/100g)	2398.08±72.195	-
4	IC50	144.43±6.673	Keep

Flavonoid Levels

The AlCl₃ colorimetric method was used to determine the flavonoid content, and a UV-visible spectrophotometer was used to read the absorbance value of the flavonoid content in the test sample. Flavonoids contain conjugated aromatic systems and have strong absorption bands in the ultraviolet and visible spectral regions (Harborne, 1987). The principle of determining the content of flavonoids by the aluminum chloride method is that aluminum chloride with a ketone group on the C-4 atom forms a complex with aluminum chloride with a hydroxyl group on the adjacent C-3 or C-5 atom (Figure 4) atoms, flavones and flavonol groups (Azizah *et al.* 2014).

This test uses quercetin as a standard solution or standard curve because quercetin is one of the flavonol type compounds that has a keto group on C-4 and a member of the flavonoid group. Quercetin is commonly used as a standard because it is widely available and has a high level of purity (Azizah *et al.*, 2014). In addition, quercetin has a strong absorption peak at a wavelength of 435 nm

making it easy to detect using UV-Vis spectrophotometry. Flavonoid levels in methanol extract *Genep basa* and *Genep basaca* can be seen in Table 2.

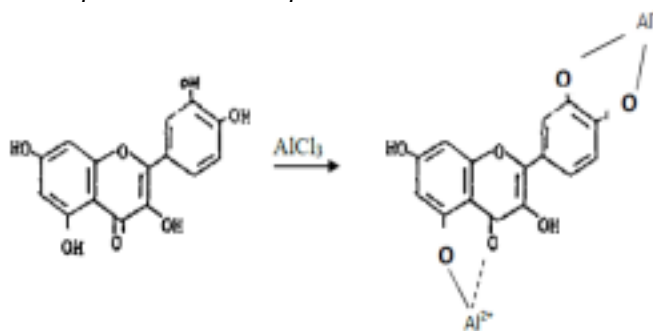


Figure 5. Formation of quercetin-aluminum chloride complex compounds
(Source: (Azizah *et al.*, 2014))

The results of quantitative test research on flavonoid levels obtained the results of flavonoid levels in methanol extract *Genep basa* amounted to 1427.90 ± 60.46 mg QE/100g. This is due to the composition of the seasoning *Genep basawhich* mostly contains flavonoid compounds. If several of these ingredients are combined, there will be a combination of two or more compounds that cause high levels of flavonoid compounds. This happens because the active compounds are synergistic. Flavonoids have antioxidant activity as free radical catchers and absorbers of singlet oxygen formation (O-) (Trianggadewi, 2010). In addition, flavonoids are also used as antibacterial, antiviral, anti-inflammatory, antiallergic and anticancer (Neldawati *et al.*, 2013).

Tannin levels

Tannins are complex chemical compounds consisting of several polyphenolic compounds that form complex compounds with Fe^{3+} ions. Tannins will suppress the process of lipid peroxidation so as to prevent hypercholesterolemia (Noer *et al.*, 2018). Tannin content testing using UV-Vis spectrophotometry method with Folin Denis reagent. The tannin standard used was tannic acid, which can be used as a comparison when measuring total tannin content since one of the tannin groups is hydrolyzed (Supriyanto, 2011).

Tannin testing will be reacted with denis folin reagents and sodium carbonate which is then read on a Uv-Vis spectrophotometer. This reagent contains phosphomolibic acid which will be reduced to molybdenum. When a material is reduced, the solution becomes blue and absorption is measured in the visible light region. Tannins act as reducing agents, the more tannins contained in a material, the more phosphomolybdate reduced to molybdenum. This reaction will cause the blue color formed to intensify and the measured absorption value is also greater (Kharismawati *et al.*, 2009). The addition of Na_2CO_3 aims to create an alkaline atmosphere so that the folin denis reduction reaction by the hydroxyl group of the polyphenol in the sample will form a blue molybdenum-tungsten complex (Ulfasari, 2021). Tannin content in spice methanol extract *Genep basa* and *Genep basaca* can be seen in Table 2.

Tannin levels tested on methanol extract *Genep basa* obtained a result of 3277.05 ± 65.33 mg TAE/100g. Tannins have a large biological role because of their function as protein depositions and metal agents. Therefore, tannins are

predicted to act as biological antioxidants by donating electrons to oxidized compounds, thereby inhibiting the oxidation process and stabilizing free radicals (Malangngi *et al.*, 2012). Tannins can also bind to free radicals and reactive molecules, stabilizing them, and preventing them from causing damage to cells.

Total Phenol Levels

Phenolic compounds have one (phenol) or multiple (polyphenol) phenolic rings, which are hydroxyl groups attached to an aromatic ring, making them susceptible to oxidation by donating hydrogen atoms to free radicals. Phenolic compounds have potential as antioxidants due to their ability to form stable phenoxy radicals during oxidation reactions (Dhurhanian and Novianto, 2019). A test for total phenolic content using the Folin-Ciocalteu (F-C) reagent reacts with phenolic compounds to produce a blue color that can be measured with a spectrophotometer at a wavelength of 760 nm. The blue color in the solution is caused by molybdenum metal (Mo(VI)) in the reagent complex, which is reduced to Mo(V) by electron donation from the antioxidant.

Standard raw solutions use gallic acid or caffeic acid. Total phenolic levels are calculated using gallic acid as the standard standard, so the results use mg units of gallic acid equivalent per g extract (Berker *et al.*, 2013; Vázquez *et al.*, 2015). Gallic acid is more widely used as a phenol standard because it is abundant in the food matrix (Pereira *et al.*, 2018). If the F-C reagent is stable, it can be protected from reducing agents and light when it is dissolved. The basic mechanism of the F-C method of measuring polyphenols in natural materials is that oxidation/reduction reactions with phenolic groups will be oxidized and metal ions will be reduced (Agbor *et al.*, 2014). Total phenol content in methanol extract *Genep basacan* be seen in Table 2.

The results of quantitative test of total phenol compound levels found that *base genep* methanol extract had total phenol compound levels of 2398.08 ± 72.195 mg GAE / 100g. The combination of several ingredients that have total phenol content also affects total phenol compound levels. These compounds will be synergistic so that they are expected to increase the levels of total phenol compounds in the *genep base*.

IC50

Antioxidants are amounts of compounds or chemical components that inhibit or slow down the damage caused by oxidative processes (Sayuti and Yenrina, 2015). Antioxidants function by donating electrons to oxidant compounds, thus inhibiting the activity of these oxidants (Winarti, 2010). Antioxidant activity is affected by many factors, such as lipid content, antioxidant concentration, temperature, oxygen tension, and general food chemical composition (e.g., protein and water) (Sayuti and Yenrina, 2015). A commonly used method for testing the antioxidant activity of an ingredient using free radicals *1,1-diphenyl-2-picrylhydrazil* (DPPH). DPPH radical reduction depends on the number of hydroxyl groups present in the antioxidant, so this method provides an indication of the structural dependency or antioxidant ability of biological antioxidants. Measurement of antioxidant activity by DPPH method using spectrophotometric principles. DPPH compounds (in methanol) of dark purple color were detected at a visible beam length of about 517 nm.

The results of the IC₅₀ test on the methanol extract of the *genep basah* have been presented in Table 2. IC₅₀ test results show that *base genep methanol extract* is classified as medium antioxidant with IC₅₀ value of 144.43±6.673 ppm. The base *Genep methanol extract* is classified in the medium category and is characterized by test results of flavonoid value, tannin value and total phenolic value of 1427.90 ± 60.46 mg QE/100g, 3277.05 ± 65.33 mg TAE/100g and 2398.08 ± 72.195 mg respectively. GAE/100g Flavonoids, tannins and phenolic compounds are antioxidants.

CONCLUSIONS AND RECOMMENDATIONS

The results found that *base genep methanol extract* had flavonoid levels of 1427.90±60.46 mg QE/100g, tannin levels of 3277.05±65.33 mg TAE/100g, total phenol levels of 2398.08±72.195 mg GAE/100g with IC₅₀ values of 144.43±6.673 ppm including medium category. *Base genep extract* is proven to contain phytochemical compounds and has antioxidant activity so that *base genep* can be used as functional food. The levels of phenols, tannins and flavonoids found in the *base genep* also function as antibacterial so that the use of the *base genep* in food can preserve and increase the shelf life of food.

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