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Antioxidant Activity, Phenolic, and Total Flavonoid Value of Balinese Trengguli Flower (*Cassia fistula*)

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Abstract: The Balinese *trengguli* flower (*Cassia fistula*), found in the northern part of Bali, is known to contain flavonoids and phenols, one of the sources of natural antioxidants that can reduce the effects of free radicals. This study aimed to extract the powder of trengguli flower with ethanol and measure the total flavonoids and phenolics. Furthermore, the antioxidant activity of the ethanol extract of Trengguli flower (EEBTF) was investigated. The filtrate was concentrated with a rotary evaporator after soaking the Trengguli flower powder in ethanol. Total phenolic and flavonoid levels were measured using UV-Vis spectrophotometry. Diphenyl-1-picrylhydrazyl (DPPH) technique was used to determine antioxidant activity. This study shows that Balinese trengguli flower extract has the highest antioxidant activity for a concentration of 100 mg/L with a DPPH radical inhibition percentage value of 87%. In this research, the ethanol extract of the Balinese trengguli flower has secondary metabolites of flavonoids and phenolics. The ethanol extract of Balinese trengguli flower has a flavonoid content of 8.35 mg QE/g and a total phenolic content of 8.12 mg GAE/g.

Keywords: Antioxidant; DPPH; Phenolic; Flavonoid; *Cassia fistula*.

Introduction

Free radicals are unstable molecules or molecular fragments with one or more unpaired electrons that can damage cell membrane lipids, DNA, and proteins, resulting in various degenerative diseases [1]. In addition, free radicals in the body can also cause damage to cells and tissues, which can stimulate organ damage, ultimately triggering chronic diseases [2]. Although the human body can carry out natural defences in overcoming the increase in free radicals within normal limits, the increase in free radicals will result in the increased pathogenesis of several diseases. These free radicals can be overcome using antioxidant compounds [3].

Antioxidants can counteract free radicals that can cause damage to cells and biomolecules, leading to degenerative diseases [4]. Through electron transfer, antioxidants can inhibit oxidation reactions by binding with free radicals [5]. Naturally, the body can produce antioxidants in insufficient quantities to protect the body's cells from the abundance of free radicals. Endogenous antioxidants can be preventive or inhibitory and repair radical damage [6]. Therefore, antioxidants are needed from outside, such as food [7]. Synthetic antioxidants cause carcinogenic effects [8]. High doses of synthetic antioxidants result in DNA damage and cause premature aging. BHA (Butylated hydroxyanisole) and BHT (butylated hydroxytoluene) caused adverse effects on the liver and carcinogenesis in animal studies [9]. The research results have triggered many studies to look for natural antioxidants found in plants or herbs. Natural antioxidants (polyphenols and carotenoids) have anti-inflammatory, anti-ageing, anti-atherosclerosis, and anti-cancer properties [10].

Various plant materials, such as grains, fruits, vegetables, herbs, and spices, are sources of natural antioxidants. One source of natural antioxidants is the Bali trengguli flower. The Balinese *trengguli* plant is often used by the community as an anti-cholesterol herbal medicine, treats constipation, and is anti-inflammatory [11]. Many Balinese *trengguli* flowers are made into tea for easy consumption. Trengguli contains flavonoids, alkaloids, tannins, phenols, glycosides, steroids, and terpenoids which have pharmacological activities such as antibacterial, anti-fungal, antidiabetic, antioxidant, anti-inflammatory, antipyretic, analgesic, hepatoprotective, hypolipidemic and antiparasitic [12]. Phenolic or polyphenols and flavonoids are plants' most significant class of secondary metabolite compounds [13]. Phenolic compounds are essential antioxidants to prevent and treat degenerative diseases and cancer [14]. Flavonoids also can ward off free radicals. In addition, it is also able to inhibit lipid oxidation. Flavonoids have antioxidant, antibacterial, anti-viral, and anti-cancer activities [15].

In antioxidant testing, the advantages of the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method include the fact that the method is simple, easy, and requires few samples and reagents [16]. The maceration extraction method is often chosen because it has advantages like cheapness, ease of work, and minimising damage to active compounds. The solvent used in the maceration process will affect the yield,

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total phenolic and flavonoid content and antioxidant activity using the DPPH method. Using an ethanol solvent in the extraction process shows high *phytocompound* concentrations [17]. Other studies have also demonstrated that the ethanol solvent used in the maceration process of the *telang* flower (*Clitoria ternatea L.*) can produce extracts with phenolic and flavonoid content. The flavonoid content of the extract of Flower *telang* (*Clitoria ternatea L.*) is and has antioxidant activity in the high category [18]. Another study that used n-hexane solvent in the maceration process did not produce phenolic compounds and flavonoids [19]. Thus, this study aims to perform antioxidant activity and the maceration extraction of Balinese trengguli flower powder using ethanol solvent and measure the total flavonoid and phenolic content contained in ethanol extract of Balinese trengguli flower (EEBTF) using UV-Vis spectrophotometric method.

Research Method

Preparation of Balinese Trengguli Flower Extract

Tools and materials in this study used a UV-Vis spectrophotometer (SHIMADZHU®), a Rotary vacuum evaporator (Eyela®), a blender, and a 60 mesh sieve. Balinese *trengguli* (*Cassia fistula*) flower simplisia powder obtained from Sangsit Village, Buleleng Regency, Bali Province, 100% ethanol (Merck), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich), gallic acid (Merck), Folin-Ciocalteu reagent (Merck), quercetin (Sigma-Aldrich), demineralised water, sodium carbonate (Merck), and aluminium chloride (Merck). All reagents used were of analytical grade. 1000 g of Balinese trengguli flower powder was soaked with 1 L of ethanol for one day. Next, it is filtered, and the filtrate is stored while the dregs are soaked again with 1 L of ethanol. If the pulp is brown, then the maceration process is stopped. Furthermore, a rotary evaporator collected the filtrate obtained for the concentration process. The extract yield was calculated using the equation [21]

$$\% \text{ Yield} = \frac{\text{weight of extract}}{\text{weight of powder}} \times 100\%$$

Determination of Total Flavonoid Level

Quercetin was weighed as much as 25 mg and dissolved with 25 mL of ethanol. The standard quercetin solution was made in the 10 - 50 mg/L concentration range. Furthermore, each concentration of quercetin was taken as much as 1 mL and added with 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M CH_3COONa, and 2.8 mL of distilled water, then each was incubated for 22 minutes. Next, the absorbance was measured using a UV-Vis spectrophotometer at 444 nm. Then, 25 mg of ethanol extract of Balinese trengguli flower (EEBTF) was weighed and dissolved with 25 mL of ethanol until a concentration of 1000 mg/L was obtained. Then, given the same treatment as the standard solution of quercetin [20].

Measurement of Total Phenolic Level

A quantity of EEBTF was put into a volumetric flask, added with 0.4 mL of Folin-Ciocalteu reagent and left for 8 minutes. After that, it was added with 4 mL of 7% Na_2CO_3 and marked with distilled water. The incubation process was carried out within 2 hours. Then, the absorbance measurement of the solution was done at 765 nm. The blank used was distilled water and Folin-Ciocalteu reagent [21].

Antioxidant Test

EEBTF test sample solutions were prepared in concentrations of (20, 40, 80, and 100) mg/L. The reaction mixture consisted of 1 mL of 6 x 10-5 M DPPH solution and 40 µL of methanol solution containing the test solution. After incubation for 20 minutes at 37oC, the absorbance of the reaction mixture was measured at a wavelength of 515 nm using a spectrophotometer to obtain the absorbance value. A blank sample with 40 µL of methanol in DPPH solution was prepared and measured at the same wavelength. The experiment was conducted with three repetitions (T1, T2, T3). Antioxidant activity was calculated using the formula. Then, a flower graph was made between sample concentration (x-axis) and percentage inhibition (y-axis). The IC_{50} value was calculated using the regression equation formula [19].

$$\% \text{ Inhibition} = \frac{\text{Abs. blank} - \text{Abs. sample}}{\text{Abs. Blank}} \times 100\%$$

Result and Discussion

Extraction of Bali Trengguli Flower

Balinese trengguli flower powder was extracted by maceration. The advantages of maceration are that the procedure is simple and fast and can maximally remove bioactive compounds from plants [22]. Table 1 shows that the yield of ethanol extract of Bali *trengguli* flower (EEBTF) is 4.0%. The yield of ethanol extract of Bali trengguli flower in this study is small. The results indicate that the content of active compounds in teak leaves is also tiny.

Furthermore, the extract was tested for antioxidant activity using the DPPH method and determined the total flavonoid and phenolic content. This study used ethanol solvent for the maceration process because it can extract non-polar and polar active compounds. In addition, other studies that also used ethanol in the extraction process showed high phytocompound concentrations. In many studies, ethanol solvent was combined with aqueous solvent to maximise antioxidant activity, flavonoid and total phenolic content. For example, a larger ratio of ethanol solvent was combined with a smaller ratio of water solvent [23,24].

Table 1. EEBTF Yields

Extract type	Weight of powder (g)	Weight of extract (g)	Yield (%)
Ethanol extract of Bali *trengguli*	1000	40	4.0

flower

Determination of Total Flavonoid and Phenolic Levels

This study measured total flavonoid and phenolic levels in EEBTF using the UV-Vis spectrophotometric method. Quercetin standard was used in the 10-50 mg/L concentration range to calculate the entire flavonoid content. Quercetin was selected as a standard because it effectively counteracts free radicals [23]. Figure 1 shows that the linear regression equation on the quercetin standard calibration curve is y = y = 0.0164x - 0.1502; R^2 = 0.9206.

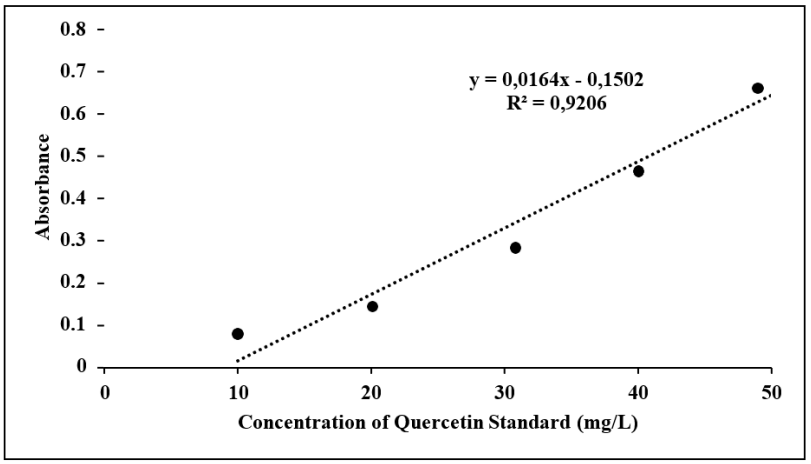

Figure 1. Quercetin Standard Curve

Table 2. EEBTF Flavonoid Levels

Extract type	Absorbance	Total flavonoid levels (mg QE/g)
Ethanol extract of Bali *trengguli* flower	1.216	8.35

The total flavonoid content in EEBTF was 8.35 mg QE/g (Table 2). Polar ethanol will easily attract flavonoid compounds, which are also polar. Flavonoids are polar compounds because they have several unsubstituted hydroxyl groups. This flavonoid compound can be an anti-microbial, wound infection drug, anti-fungal, anti-viral, anti-cancer, and anti-tumour. Flavonoids are a group of phenolic compounds with antioxidative properties that prevent damage to cells and their cellular components by reactive free radicals. In another study, the ethanol extract of flower kenanga (Cananga odorata) has more excellent flavonoid content than ethyl acetate extract [25]. In another report, ethanol extract of P. emblica leaves had the highest total flavonoid content, followed by ethyl acetate and n-hexane extracts [26]. Some of these reports indicate that ethanol solvent can be used to extract total flavonoids from plants maximally. The gallic acid standard was used in the 10-50 mg/L concentration range to measure phenolic content. Gallic acid was chosen as the standard because it is a natural phenol, stable and cheap. In addition, gallic acid is very effective in forming complex compounds with Folin-Ciocalteu reagent [27]. Figure 2 shows that the linear regression equation on the gallic acid standard calibration curve is y = 0.0093x + 0.042 with an R^2 value = 0.8633.

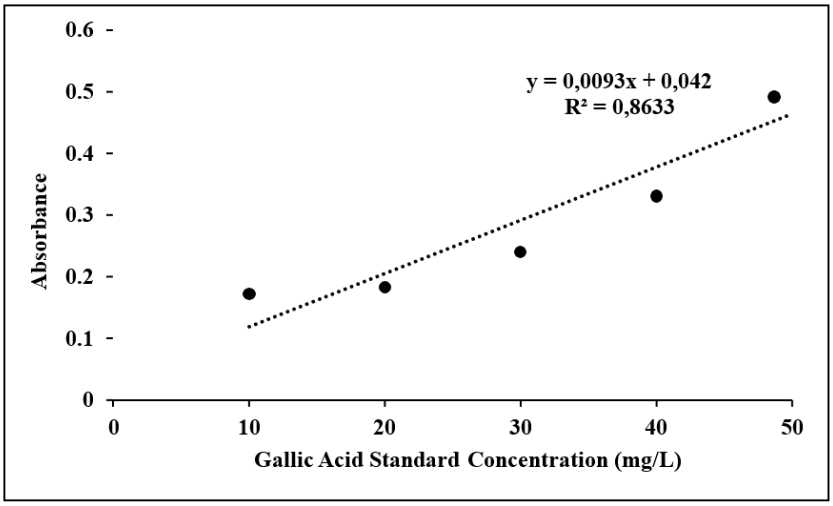

Figure 2. Calibration Curve of Gallic Acid Standard Solution

Table 3. EEBTF Phenolic Levels

Extract type	Absorbance	Total phenolic levels (mg GAE/g)
Ethanol extract of Bali *trengguli* flower	0.7265	8.12

Based on Table 3, it can be seen that the total phenolic content in EEBTF is 8.12 mg GAE/g. In another study, ethanol extract of *kersen* leaves had an entire phenolic content of 1.163 mg QGA/g extracted using the maceration method and 2.53 mg QGA/g extracted using the *sokletasi* method [28]. In addition, ethanol solvent can also be used to extract total phenolic compounds in flower *telang* with levels of 19.43 ± 1.621 GAE (mg/g sample) [29]. Some of these reports indicate that ethanol solvents can be used to maximally extract total phenolics from plants.

Antioxidant Test Using the DPPH Method

DPPH is a radical compound that can indicate the reduction process of antioxidant compounds. The principle of testing is by reacting antioxidant compounds with free radical compounds. The working principle of the DPPH method is the presence of hydrogen atoms from antioxidant compounds that bind to free electrons in radical compounds, causing changes from free radicals (*diphenylpicrylhydrazyl*) to non-radical compounds (diphenylpicrylhydrazine). The concentration of ethanol extract of Balinese trengguli flower used in an antioxidant test using the DPPH method is 20, 40, 80, and 100 mg/L. In the antioxidant test using the DPPH method, vortexing for 1-minute aims to maximise mixing or homogenisation between the extract test solution and DPPH solution [15].

On the other hand, the incubation process for 30 minutes aims to maximise the DPPH inhibition reaction by the test solution. Absorbance measurement in the antioxidant test was done at 515 nm because DPPH can provide strong absorption. The DPPH method has the principle that the hydrogen donation of electrons from the test solution of Bflower *telang* ethanol extract to DPPH causes a decrease in the absorbance value of DPPH [18]. The color change indicates the antioxidant activity of the extract test solution. The DPPH method has advantages, including simplicity and speed, and it does not use many chemical reagents.

Table 4. Antioxidant Test of EEBTF

Concentration (mg/L)	The absorbance of the Test Solution				Antioxidant Activity (%)
	T1	T2	T3	Mean	
20	0.793	0.801	0.814	0.802	65
40	0.771	0.765	0.741	0.759	73
80	0.762	0.521	0.533	0.605	80
100	0.442	0.439	0.437	0.439	87

Based on Table 4, the antioxidant activity of the ethanol extract of Balinese trengguli flower increased from the lowest concentration of 20 mg/L by 65% to the highest concentration of 100 mg/L by 87%. Thus, it can be determined that the concentration of ethanol extract of Balinese trengguli flower that produces the highest antioxidant activity is 100 mg/L. The increasing percentage inhibition indicates that increasing the extract concentration will also increase the percentage inhibition. Many studies reported that the increase in percentage inhibition, which is proportional to the increase in antioxidant activity, will be followed by an increase in the concentration of the extract or test solution [30-32]. The rise in percentage inhibition and an increase in the concentration of ethanol extract of the Balinese trengguli flower is due to more antioxidant compounds in the extract that can counteract DPPH free radicals.

In this research, the ethanol extract of the Balinese *trengguli* flower has secondary metabolites of flavonoids and phenolics. Hydrogen atoms from phenolic compounds will be donated to DPPH. The potential or effect as a flavonoid or phenolic antioxidant is due to its ability to overcome oxidative stress and reactive oxygen species (ROS) [31]. Flower trengguli (*Cassia fistula*) has also been studied as antiobesity and antidiabetes [33], antibacterial [34], and anti-cholesterol [35]. The potential of ethanol extract from Balinese trengguli flower needs to be further investigated as antihypertensive, anti-hyperuricemia, and antihyperglycemia. Furthermore, the standardisation of ethanol extract of Balinese trengguli flower must also be done to meet the requirements of Standardised Herbal Medicine.

Conclusion

In this study, Balinese *trengguli* flower extract has the highest antioxidant activity for a concentration of 100 mg/L with a DPPH radical inhibition percentage value of 87%. In this research, the ethanol extract of the Balinese trengguli flower has secondary metabolites of flavonoids and phenolics. The ethanol extract of Balinese trengguli flower has a flavonoid content of 8.35 mg QE/g and a total phenolic content of 8.12 mg GAE/g.

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