



COMPARISON OF THE ANTIOXIDANT ACTIVITY OF TRADITIONAL HERBAL LOLOH FROM BALI AS AN IMMUNITY BOOSTER

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ABSTRACT

Loloh is a local wisdom product of the Balinese people that has been passed down from generation to generation, processed from plant leaves. Consumption of loloh is utilized by the community as a preventive measure to prevent disease and increase body immunity. This has great potential to be applied in facing the growing disease trends in the future, starting from infectious diseases and non-infectious diseases (degenerative). Herbal drinks in general made from ginger, turmeric and temulawak have been widely studied. However, until now, the antioxidant power of loloh from Bali is not yet known. The purpose of this study was to determine the comparison of the antioxidant power of traditional Balinese loloh. This study used a descriptive explorative and experimental design by applying the DPPH (2,2-diphenyl-1-picrylhydrazyl) method to determine the antioxidant power of each loloh sample by looking at its IC₅₀ value. The data collection technique uses purposive sampling. The number of samples studied in this study was ten samples including loloh cemcem, beras kencur, rosella, telang, mengkudu, kelor, kunyit asam, temu kunyit, kunyit asam sirih, and cincau hijau. Data analysis was conducted using regression equations. The results of IC₅₀ measurements showed that the traditional loloh that had very strong antioxidant activity was loloh kelor leaves with an IC₅₀ value of 98 ppm, weak antioxidant activity was possessed by loloh rosella flower tea and cincau leaves with IC₅₀ values of 168 ppm and 198 ppm while very weak antioxidants were possessed by loloh telang flowers, cemcem, beras kencur, mengkudu, kunyit asam, temu kunyit, and kunyit asam sirih with IC₅₀ values > 200 ppm.

Keywords: antioxidants; DPPH; IC₅₀; immunity; loloh

How to cite (in APA style)

Widayanti, N. P., Laksmita W., A. S., Apriyanthi, D. P. R. V., & Widnyani, N. M. (2024). Comparison of the Antioxidant Activity of Traditional Herbal Loloh from Bali as an Immunity Booster. *Indonesian Journal of Global Health Research*, 6(S6), 1273-1280. <https://doi.org/10.37287/ijghr.v6iS6.5319>.

INTRODUCTION

Bali is one of the islands in Indonesia that is famous for its cultural heritage from generation to generation. The abundant biodiversity encourages people to utilize the potential of these plants traditionally. Its existence in a tropical climate region causes this area to be overgrown with various plants that have phytopharmaceutical potential. One of the uses of plants in Bali is making loloh drinks. Jamu is a traditional Indonesian herbal medicine that has been practiced for centuries in Indonesian society to maintain health and treat diseases. In Bali, this traditional herbal medicine is known as loloh which is a product of local wisdom of the Balinese people that has been passed down from generation to generation which is believed to be able to prevent disease and increase body immunity. This drink is a herbal medicine processed from plant leaves that are believed to have medicinal properties. Consumption of loloh is used by the community as a preventive measure to prevent various diseases such as headaches, diuretics, diabetes, heartburn, increase appetite and body immunity (Sujarwo et al., 2016).

The basis used by the Balinese people in making this loloh drink comes from the Usada Taru Pramana palm leaf manuscript which contains traditional knowledge about the diversity of plant species and their use to treat various diseases (Arsana, 2019). Although there are many modern medicines, herbal medicine is still very popular in both rural and urban areas.

Traditional herbal medicine has become a healthy lifestyle trend and a natural immune booster during the pandemic. Traditional herbal medicine products made from ginger, turmeric and temulawak are known to have active ingredients that play a role in health (Kusumo et al., 2020; Elfahmi et al., 2014). However, until now, the antioxidant power of traditional herbal medicine in the form of loloh originating from Bali is not yet known.

Previous studies have stated that the active ingredients of the pegagan plant include alkaloids, saponins, flavonoids, steroids, and triterpenoids which function to increase immunity (Sutardi, 2016). Research conducted by Hasim et al. (2019) stated that the ethanol extract of starfruit leaves (*Averrhoa bilimbi*) has the potential as an antioxidant and anti-inflammatory. Ruhardi et al. (2021) stated that the active substance content in sembung leaves (*Blumea balsamifera* L.) is in the form of flavonoids which act as antioxidants. Suryanita et al. (2021) stated that the ethanol extract of Bali orange peel (*Citrus maxima* Merr.) contains active substances in the form of flavonoids, saponins, alkaloids, triterpenoids/steroids and tannins so that it has antioxidant activity. Ardiansyah et al. (2016) stated that the process of processing katuk leaves on a household scale such as boiling, steaming, and microwave treatment can affect their antioxidant content and activity. Buanasari et al. (2018) stated that the presence of antioxidant groups in the form of phenols in tamarind leaf extract has the potential as a source of beneficial natural antioxidants. Previous research stated that the ethanol extract of cemcem/forest kedondong leaves (*Spondias pinnata* (L.) Kurz.) Has antioxidant activity through various tests (Azizah et al., 2019).

Bali is divided into nine regencies/cities consisting of Jembrana, Tabanan, Badung, Gianyar, Klungkung, Bangli, Karangasem, Buleleng and Denpasar. Each region has its own typical loloh drink. Based on the results of a preliminary survey, the types of loloh drinks that are famous in Bali include loloh cemcem, beras kencur, rosella, telang, mengkudu, kelor, kunyit asam, temu kunyit, kunyit asam sirih, and cincau hijau. The plants used as ingredients for making loloh are types of plants that are thought to be rich in secondary metabolite content that acts as natural antioxidants for the body. The secondary metabolites that act as antioxidants include phenolic compounds, flavonoids and tannins. Antioxidants are known to be able to fight free radicals by donating the electrons they have.

As time goes by, the challenges faced by the world of health today are the development of various diseases, both infections that can come from microorganisms (bacteria, fungi, viruses and parasites) and non-infectious diseases (degenerative) caused by unhealthy lifestyles. To prevent the emergence and development of various diseases, the body's immunity must be maintained properly. One effort to increase the body's immunity is to consume sources of antioxidants such as traditional loloh. Traditional loloh produced by the community is made conventionally. The storage process cannot last long because it does not contain preservatives. The process of testing the content of the loloh has never been carried out so that the community does not know for sure the content of secondary metabolites and the antioxidant power contained in it. In addition, until now the antioxidant power of loloh from Bali is not known. The aim of this research is to explore and test the comparison of the antioxidant power of traditional loloh which is efficacious as a natural defense fort to increase the body's immunity.

METHOD

This study used a descriptive explorative and experimental design aimed at exploring and comparing the antioxidant power of traditional loloh drinks typical of Bali. The stages of the method used in this study are as follows: 1)Preliminary Survey: This stage is the beginning of the research activity where the researcher together with the team conducted a survey of

traditional loloh drinks typical of the nine districts/cities in Bali. In this activity, the researcher prepared a questionnaire and conducted interviews with several residents in each region. 2) Sampling of Traditional Loloh Drinks: At this stage, the researcher together with the team took samples from the location using a sample box containing dry ice so that the samples would not be damaged when they arrived in the laboratory. 3) Laboratory Testing: The sample testing process in the laboratory consists of several stages, including: a) Sample preparation: After sampling, the researcher labeled each sample. Furthermore, the samples obtained were evaporated with a rotary evaporator because the largest component in traditional Balinese loloh drinks is water. Water is a solvent that must be evaporated in order to obtain a concentrated sample. b) Determination of Antioxidant Power (IC50) by DPPH (2,2-diphenyl-1-picrylhydrazyl).

Antioxidant activity testing was carried out on samples and quercetin standards as a comparison. Antioxidant activity testing was carried out using the DPPH radical scavenging method (Buanasari et al., 2018). Traditional loloh sample solutions were made with concentration variations of 50, 100, 150, 200, and 250 ppm. Furthermore, 1 ml was taken from each sample and 3 ml of 0.0040% DPPH solution in methanol was added. Then this mixture was shaken and stored in a dark room for 30 minutes so that the reaction was perfect, then the absorbance was measured with a UV-Vis spectrophotometer at a wavelength of 520 nm. The test was carried out with 3 repetitions and the absorbance obtained was then calculated for the % reduction using the formula:

$$\% \text{ Inhibisi} = \frac{\text{Absorbansi kontrol} - \text{Absorbansi sampel}}{\text{Absorbansi kontrol}} \times 100\%$$

Next, a linear regression curve is determined between sample concentration and average inhibition percentage. Determination of antioxidant activity is done by calculating the inhibition concentration value (IC50). The IC50 value is obtained from the equation $y = ax + b$ on the linear regression curve of the relationship between concentration (x) and the percentage of attenuation (y). The IC50 results of the sample are said to be active as an antioxidant if they have an IC50 <200µg/ml. The data were analyzed descriptively qualitatively. The data obtained were primary data from the results of phytochemical screening of traditional loloh samples. The data obtained from the screening results were made in the form of tables and graphs, then the results were described. The results of the antioxidant activity test were known by making a linear regression curve until the IC50 value of the sample was known.

RESULT

The results of the comparative test of ten traditional Balinese loloh drinks can be divided into two, namely the comparison of color observations and antioxidant activity indicated by the IC50 value.

Table 1.
Comparison of Observation Results of Traditional Balinese Loloh Color

Sample	Loloh's Name	Contents	Color Observation
L1	Cemcem	Forest kedondong leaves (cemcem)	Green
L2	Saffron-colored rice	Rice and galangal	Light yellow
L3	Rosella	Roselle flower	Red
L4	Telang	Butterfly pea flower	Purple
L5	Noni	Noni fruit	Clear yellow
L6	Moringa	Moringa leaves	Green
L7	Turmeric and tamarind	Turmeric and tamarind	Yellow
L8	Turmeric	Curcuma and turmeric	Chocolate
L9	Turmeric, tamarind, betel leaf	Turmeric, tamarind and betel leaves	Deep yellow
L10	Green grass jelly	Green cincau leaves	Green

IC50 is a parameter that can be used to measure the antioxidant power of a sample. The results of the antioxidant activity test of each traditional loloh sample are presented in Figure 1.

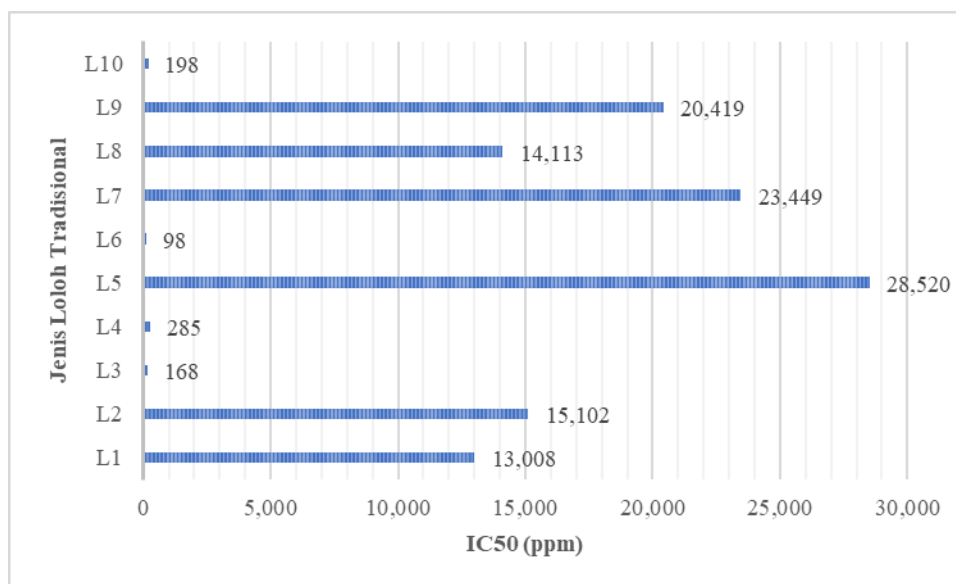


Figure 1. Comparison of Antioxidant Activity of Traditional Balinese Loloh

DISCUSSION

Comparison of antioxidant power of traditional Balinese loloh

Loloh is a type of traditional medicine consumed by the community not only to prevent a disease (preventive) but sometimes used as an effort to treat certain diseases (curative). Loloh sold in the community varies in type, ingredients, taste and color. Color is one of the attractions for buyers to loloh products. Attractive colors and aromas can influence consumer interest in consuming them. Kusumawati & Yogeswara (2020) stated that the color of traditional loloh affects the level of consumer preference to consume it. The color of loloh that does not have added sweeteners gives a dark green color while the addition of coloring changes the color of the loloh. The difference in color influences the acceptance of consumer preferences for traditional loloh.

Judging from the results of the antioxidant activity test of the ten types of traditional Balinese loloh, it was found that moringa loloh has the strongest antioxidant power in warding off free radicals with an IC50 value of 98 ppm in the strong category. The antioxidant content in moringa includes vitamin C, polyphenols, flavonoids and carotene. Where vitamin C is a natural antioxidant that has the highest antioxidant activity and which functions as an inhibitor to inhibit oxidation by reacting with reactive free radicals to form relatively stable non-reactive free radicals. The β -carotene content in moringa leaf extract also protects lipid membranes from peroxidation and at the same time stops the chain reaction of free radicals (Tjong et al., 2021). The content of flavonoids and polyphenols can significantly increase Superoxide Dismutase (SOD) and catalase so that it can increase antioxidant defenses in the body (Adriana et al., 2019). The antioxidant compounds contained in moringa leaves are beta carotene and flavonoids. Beta carotene is a red-orange, orange, and yellow pigment found in moringa leaves. Beta carotene is insoluble in water, soluble in fat, and easily damaged due to oxidation at high temperatures. Beta carotene can reduce the risk of cancer and heart disease (Kusbandari and Susanti, 2017). The main flavonoid found in *Moringa oleifera* is quercetin. Quercetin is a strong antioxidant compound found in *Moringa* leaves. Quercetin comes from the flavonol group with the IUPAC name 3,3',4',5,7-pentahydroxyflavanone. The structural formula of quercetin is C₁₅H₁₀O₇. Quercetin is one type of flavonoid that is abundant with a

citron yellow aglycone and one type of flavonoid that is abundant. Quercetin has broad bioactivities such as antioxidant, anti-inflammatory, antiviral, antibacterial, anticarcinogenic, liver-protecting, and antiplatelet. In addition, quercetin has a cardioprotective effect against myocardial ischemic damage. This compound can inhibit the activity of angiotensin converting enzyme, increase blood vessel relaxation, and reduce oxidative stress (Grande et al, 2016).

Research by Alimsyah et al. (2020) on the antioxidant test of moringa leaf extract using the DPPH method showed that moringa leaf extract is included in strong antioxidants because it has an IC₅₀ value of 79 ppm. Moringa leaf extract contains strong antioxidant compounds such as flavonoids, namely quercetin and beta carotene which have many H atoms that can be donated to neutralize oxidants so that they will provide high antioxidant activity. Research by Fachriyah et al., (2020) on the antioxidant activity test of moringa leaf ethanol extract using the DPPH method showed that quercetin contained in moringa leaf ethanol extract has an IC₅₀ value of 3.5076 ppm, which means that moringa leaf ethanol extract has very high antioxidant activity.

The next type of loloh that has quite strong antioxidant power is rosella flower tea with an IC₅₀ value of 168 ppm. This study is supported by research conducted by Dwiyanti and Nurani (2014) which states that rosella tea has strong antioxidant power because it contains secondary metabolite compounds in the form of anthocyanins. One of the important contents of herbal rosella flowers is anthocyanin compounds which are effective as antioxidants (Nurnasari & Khuluq, 2017). The antioxidant activity of anthocyanins is greater when compared to a-tocopherol (vitamin E), ascorbic acid, and beta carotene (Malinda & Syakdani, 2020). Anthocyanin compounds are able to neutralize free radicals. The antioxidant activity of herbal rosella at a dose of 1000µg is able to inhibit the effects of superoxide anion radicals by 70 ± 80% (Nurnasari & Khuluq, 2017). All parts of the herbal rosella plant contain potential compounds with antioxidant activity. The part of the plant extracted with water that has the highest antioxidant activity is in the calyx and the lowest is in the stem (Table 1). This is because the calyx contains vitamin C or ascorbic acid (141 mg/100 g), anthocyanin (2.52 mg/100 g), β -carotene (1.88 mg/100 g), lycopene (164 µg/100 g), polyphenols and other water-soluble antioxidant compounds (Octaviani & Rahayuni, 2014).

Green grass jelly leaf extract also has quite strong antioxidants with an IC₅₀ value of 198 ppm. The antioxidant activity of green grass jelly is due to the content of phenol compounds, which generally function to prevent damage due to oxidation reactions in pharmaceuticals and foods. Antioxidants are contained in food ingredients naturally and are useful as protection against damage to food ingredients due to oxidation reactions. Table 2 states that the antioxidant activity without the addition of kappa carrageenan has a value of 1.16% and continues to increase with increasing kappa carrageenan. The hydroxyl groups in carrageenan are large in number, so that the double helix structure can be formed higher and can protect antioxidant compounds and during the cooking process from heat and oxygen (Atmaka et al., 2020).

Phytochemical screening of *C. barbata* was conducted by various researchers to determine the content of secondary metabolite compounds found in green grass jelly plants including phenols, alkaloids, flavonoids, tannins, saponins, triterpenoids, steroids, and coumarins. Most of the compounds that can be isolated from *C. barbata* are alkaloids, such as tetrandrine, limacine, thalrugosine, homoaromoline, cycleapeltine, (+)-Cycleabarbatine, and (-)-2'-Norlimacine (Guinaudeau et al., 1993; Lin et al., 1993). The structure of the alkaloid compounds that were successfully isolated from *C. barbata* include Tetrandrine and

thalarugosine which are stereoisomers of limacine which were successfully isolated from the roots of *C. barbata*. Homoaromoline has been previously isolated from ten types of plants, but this is the first isolation from *C. barbata* as well as cycleapeltine which is its stereoisomer with antimalarial and cytotoxic activity (Lin et al., 1993). Isolates from *C. barbata* roots, (+)-Cycleabarbata and (-)-2'-Norlimacine also have the same pharmacological activity, namely antimalarial and cytotoxic (Ramadani, 2017).

The flavonoid compound group that was successfully isolated from *C. barbata* leaves such as isoquercetin has anti-inflammatory and anti-radical properties (Ismail et al., 2020). According to Kusmardiyani et al. (2014), flavonol 3-O-glycoside with 4'-hydroxyl and 6,7,8-orthodihydroxy isolated from *C. barbata* leaves is included in the flavonoid compound group. However, Kusmardiyani et al. (2014), has not confirmed the name of the compound because the position of ortho-dihydroxy is still unclear. *C. barbata* also contains tannins that can lower total cholesterol by preventing reabsorption and increasing cholesterol excretion (Tibe et al., 2018).

The type of butterfly pea flower infusion also showed weak antioxidant activity with an IC50 value of 285 ppm. The butterfly pea flower contains flavonoids, phenolics, alkaloids, and terpenoids, while the leaves contain the same components, but do not contain terpenoids but contain saponins. (Table 1). Overall, the phytochemical content of macerated, brewed, and boiled flowers is similar. However, what makes the difference is the level or concentration of phytochemicals. These results indicate that different extraction techniques on flowers do not cause differences in the types of phytochemical components, but rather cause differences in phytochemical concentrations. Research conducted by Purwanto & Sulistiyani (2022) stated that alkaloid compounds are bioactive components found in almost every part of the Butterfly Pea Flower. Research conducted by Kumar et al. (2017) showed that the water extract of flowers, leaves, and roots of Butterfly Pea Flower was dominated by alkaloid and saponin compounds. The results showed that the identified bioactive components tended to vary. According to Bribi (2018), alkaloids are likely to form salts with mineral acids (hydrochloride, sulfate, nitrate) or organic acids (tartrate, sulfamate, and maleate) in plant tissues. Alkaloid salts are generally soluble in water and dilute alcohol. This is what causes the possibility of differences in the types of bioactive components, especially alkaloids, that are successfully extracted. Meanwhile, the other six traditional lolohs, namely cemcem, beras kencur, mengkudu, kunyit asam, temu kunyit, and kunyit asam sirih have very weak antioxidant power because the IC50 value shows a value above 200 ppm. However, if the ingredients of each loloh are made in the form of extracts through extraction techniques, then these solvents will be able to draw bioactive compounds from the plants.

CONCLUSION

Based on the results of the research that has been conducted, it can be concluded that the traditional loloh that has very strong antioxidant activity is the moringa leaf loloh with an IC50 value of 98 ppm, weak antioxidant activity is possessed by the rosella flower tea and cincau leaf loloh with IC50 values of 168 ppm and 198 ppm while very weak antioxidants are possessed by the loloh of butterfly pea flowers, cemcem, beras kencur, mengkudu, turmeric and tamarind turmeric and sirih with IC50 values > 200 ppm.

ACKNOWLEDGEMENTS

The author would like to express his deepest gratitude to Bali International University which has funded this research through the UNBI Grant and various parties who have assisted in implementing this research activity.

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