

ANTIOXIDANT ACTIVITY TEST OF COMBINATION OF *Centella asiatica* LEAF EXTRACT AND MINT LEAF EXTRACT AS AN ALTERNATIVE HERBAL DRINK

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Abstract: Antioxidant compounds are vital for the body. An imbalance in the body's levels of free radicals and antioxidants will cause oxidative stress and cause disease because free radicals will damage the surrounding molecules (lipids, proteins, and nucleic acids), causing damage to tissues and cells. Today many studies are interested in using natural antioxidants in food, beverages, cosmetics, and therapies derived from plants. *Centella asiatica* is a plant often used as a source of antioxidants because it contains active compounds such as terpenoids, flavonoids, polyphenols, tannins, carotene, and vitamin C, which have antioxidant activity. In addition to containing 78% menthol, mint leaves contain resin and tannins, which are several polyphenolic antioxidants that prevent or neutralize the effects of free radicals. Antioxidants are compounds that can delay, slow down, or inhibit oxidation reactions and fight free radicals. The results showed that the antioxidant activity of the combination of *Centella asiatica* leaf extract and mint leaf extract based on the results of the analysis showed that it was included in the strong category.

Keywords: Antioxidant, Extract, *Centella asiatica*, Mint

INTRODUCTION

Tea is a drink that contains tannins and polyphenols, which is an infusion made from brewed leaves, leaf buds, or dried leaf stalks [1]. The principle of tea processing is to dry the leaf parts of the plant in the form of leaves and skin to reduce the water content in that part, most tea comes from the leaves of the tea plant, but some leaves are processed into tea from other plants [2]. Some plants whose leaves can be used for herbal teas are *Centella asiatica* leaves and mint leaves.

Exogenous antioxidants can be derived from chemical or natural substances. Naturally, various sources of antioxidants generally come from food. Examples of foodstuffs that produce antioxidants are fruits and plants, especially medicinal plants. As a source of antioxidants, medicinal plants can fight free radicals [3].

Gotu kola (*Centella asiatica*) is a plant that is often considered a weed. Its leaves are shaped like a kidney, with the base curved inwards. People use *Centella asiatica* leaves in several places as fresh vegetables, drinks, and traditional medicine [4]. *Centella asiatica* has long been used as a traditional medicine both in the form of fresh and dry ingredients and in the form of concoctions. This plant has a pharmacological effect, as evidenced by several studies that have been conducted.

In addition to containing 78% menthol, mint leaves contain resin and tannins, which are several polyphenolic antioxidants that prevent or neutralize the effects of free radicals. Antioxidants are compounds that can delay, slow down, or inhibit oxidation reactions and can fight free radicals [5]. Free radicals are highly reactive molecules because they

have one or more unpaired electrons. Excess free radicals can attack any compound and have implications for the emergence of various diseases such as heart disease, cancer, arteriosclerosis, and symptoms of aging [6].

This study aimed to determine the antioxidant content of a combination of *Centella asiatica* extract and mint leaves as an alternative herbal drink, as it is known that *Centella asiatica* and mint leaves contain bioactive compounds, which are natural antioxidants. Based on the results of several studies also show that *Centella asiatica* and mint leaves have many benefits, especially for health. Therefore, research on the antioxidant content of a combination of *Centella asiatica* extract and mint leaves as an alternative herbal drink needs to be carried out.

RESEARCH METHODS

Place and time of research

This research was conducted for three months, from June to August 2022. The research was carried out at the Udayana University Analytical Laboratory and the Bali Institute of Technology and Health Biomedical Laboratory.

Research design

The research design was completely randomized (CRD) with one positive control group and six treatment groups with four replications each. The treatment group used concentrations of 0, 5, 10, 15, 20, and 30 ppm.

Preparation phase

Extract Sample Preparation

The samples used were *Centella asiatica* and mint leaves obtained from Badung Market, Denpasar, Bali. *Centella asiatica* leaves, and mint leaves are dried in the open air until dry and ground into powder.

Preparation of extracts of *Centella asiatica* and mint leaves

Powdered *Centella asiatica* and mint leaves were then macerated with 96% methanol solution (technical) for 24 hours, then filtered using gauze and filter paper. Maceration was carried out three times until the solvent was clear. The liquid extract from the sample is then evaporated using a vacuum rotary evaporator device. This evaporation process was carried out until a viscous extract in the form of a gel was obtained.

The methanol extract obtained was then partitioned with n-hexane solvent using a separatory funnel. The fraction obtained was separated from the solvent using a rotary evaporator, and the n-hexane fraction was obtained. Furthermore, the methanol extract was added to distilled water, then partitioned with chloroform. After being separated from the solvent using a rotary evaporator, the chloroform fraction was obtained. Then the methanol extract was partitioned further with ethyl acetate, and the ethyl acetate fraction was obtained similarly.

Research Implementation Stage Isolation of Active Compounds

The ethyl acetate fraction was further analyzed by vacuum column chromatography using silica gel as the stationary phase and several eluent systems as the mobile phase. The chromatography results were collected in bottles, and the TLC test was carried out. Bottles showing spots with the same R_f value were combined and then concentrated with a rotary evaporator to obtain a thick isolate. Press-column chromatography is used to obtain sub-fractions or pure compounds. The compound obtained is purified by crystallization/recrystallization, and its boiling point is determined. Identification of compounds was carried out using infrared and UV-Vis spectrophotometry data.

Analysis of Antioxidant Capacity and Activity

Analysis of antioxidant capacity was carried out by preparing gallic acid concentration series with concentration series of 0, 5, 10, 15, 20, and 25 ppm gallic acid. The absorbance is read at a wavelength of 517 nm. IC 50 analysis was carried out to determine the sample concentration needed to inhibit 50% of DPPH free radicals. The addition of compounds suspected of having antioxidant content to DPPH will reduce the concentration of DPPH and cause a decrease in the absorbance value of DPPH when compared to the absorbance of the DPPH control, which is not added to compounds suspected of having antioxidant compounds.

Data analysis

Data analysis was carried out quantitatively using a computer statistics program (SPSS 22.0 for Windows). For the normality of the data, the Kolmogorov-Smirnov test was carried out. If the data was not normal, the Kruskal Wallis test was carried out. The Leven's Test was carried out to see the homogeneity of the variance. To see the effect of the treatment.

RESULTS AND DISCUSSION

The results of the antioxidant test analysis of the combination of *Centella asiatica* leaf extract and mint leaf extract can be seen in table 3.

Table 1 shows that the antioxidant content of *Centella asiatica* extract is included in the strong category. Table 2 also shows that the antioxidant content of mint leaf extract is also in the strong category. Tables 1 and 2 show no significant difference between the antioxidant content of *Centella asiatica* extract and mint leaf extract. From table 3, it was found that the antioxidant content of the combination of *Centella asiatica* leaf extract and mint leaf extract was included in the strong category. The smaller the IC₅₀ value of an antioxidant, the stronger the antioxidant. The IC₅₀ analysis results showed no significant difference between gallic acid concentrations. It shows that the antioxidant content in the combination of *Centella asiatica* leaf extract and gallic acid extract of mint leaves can react with peroxy and hydroperoxy free radicals formed from oxidation reactions [7]. The gallic acid radicals formed are stabilized by interacting with two hydrogen bonds at the ortho position [8]. A compound can be an antioxidant influenced by several factors, namely the number of phenol groups, the position of the –OH group in the compound, and the presence of other functional groups [9]. The gallic acid structure has a functional group –OH, capable of reacting with free radicals, thereby avoiding further oxidation processes.

The addition of peppermint extract significantly affected the test for water content, ash content, antioxidant activity, and the total polyphenol content of the resulting *Centella asiatica* leaf tea [10]. In this study, the antioxidant activity of adding peppermint extract to *Centella asiatica* leaf tea showed that the more peppermint extract, the higher the antioxidant activity. Mint leaves have antioxidant compounds such as limonene, cineole, menthone, menthol and pulegone, polyphenols (19%), carotenes, and tocopherols [11-12]. The *Centella asiatica* plant contains antioxidants such as flavonoids, polyphenols, saponins, triterpenoids, and steroids [13].

Table 1. Average series of gallic acid concentrations of *Centella asiatica* leaf extract

Gallic Acid Concentrations	Mean IC ₅₀ (ppm) ± Standard Deviation
0	89.32 ± 2.26a
5	87.85 ± 3.27a
10	88.72 ± 6.93a
15	89.36 ± 4.53a
20	85.32 ± 3.32a
25	81.37 ± 4.37a

Note: different letters in one row indicate significantly different results at the 5% test level

Table 2. Average series of gallic acid concentrations of mint leaf extract

Gallic Acid Concentrations	Mean IC ₅₀ (ppm) ± Standard Deviation
0	85.76 ± 6.36a
5	82.48 ± 3.47a
10	80.34 ± 4.38a
15	81.67 ± 7.23a
20	80.63 ± 5.47a
25	82.98 ± 3.49a

Note: different letters in one row indicate significantly different results at the 5% test level

Table 3. Average series of gallic acid concentrations of *Centella asiatica* leaf extract and mint leaf extract

Gallic Acid Concentrations	Mean IC ₅₀ (ppm) ± Standard Deviation
0	88.33 ± 3.26a
5	86.38 ± 4.27a
10	85.92 ± 7.62a
15	85.32 ± 5.28a
20	83.27 ± 2.37a
25	80.33 ± 6.37a

Note: different letters in one row indicate significantly different results at the 5% test level

Centella asiatica leaves, and mint leaves can be used as natural antioxidant drinks. Antioxidants can provide endogenous protection and exogenous oxidative stress by capturing free radicals. Antioxidants can act as radical hydrogen donors or as free radical acceptors to delay the initiation stage of free radical formation. Several plants contain antioxidant compounds mainly due to secondary metabolites such as flavonoids, so many are formulated as natural antioxidants which can be made in oral dosage forms as vitamins and topically as skin care products [14].

The flavor of herbal teas is useful for adding flavor to the loss or reduction of the aromatic compounds in tea, mostly composed of volatile alcohols, aldehydes, and ketones. Losing the original flavor of the instant tea food can be modified by

adding certain flavors or flavor enhancers like peppermint. Peppermint in the form of an oil extract peppermint has a variety of esters, notably menthyl acetate, which yields the aroma and flavor of "minty" it is distinctive and very sharp, so it is commonly used as an additional flavor in beverages such as tea and lemon [15].

Mint is an herbal plant that contains essential oils. Candy is widely used as a flavoring in food, cosmetics, and industrial pharmacy. Mint leaves can flavor culinary dishes, vinegar, jellies, and drink. Leaf content mint is 1% essential oil, 78% free menthol, and 2% menthol mixed with esters, and the rest is resin, tannin, and acetic acid. Mint leaves are often added to mixed drinks because there are aroma components such as menthone (21, 45%), isomentone (2.87%), menthofuran (1-17%), carvone, linalool, and piperitone oxide [15].

Naturally, plants contain secondary metabolite compounds has the potential to an antioxidant. *Centella asiatica* is wrong. A type of herb that benefits comprehensive and varied urine laxatives, thrush medicine, reducer heat, appetite enhancer, and including antioxidants. Besides that, *Centella asiatica* has the potential for a solid immunomodulatory response to high levels of primary antibody and secondary. The compound responsible for the potency immunomodulator is triterpene glycosides (asiatikosida). Apart from asiaticoside, *Centella asiatica* is also known to contain flavonoids, polyphenols, steroids, triterpenoids, and alkaloids are included in the class of metabolites with beneficial antioxidant activity health. This shows that *Centella asiatica* can be further exported to developed as an antioxidant agent experience [16].

Other research also states that *Centella asiatica* leaf extract contains antioxidants that can be used as an alternative to antihyperglycemic herbal drinks [17]. Herbal tea contains tannins and polyphenols, an infusion made by brewing leaves, leaf buds, or dried petioles of the Sinensis plant in hot water [18]. Mint leaves can also be used as a natural herbal drink with a high antioxidant content [19]. The combination of herbal drinks using mint leaves also has a high antioxidant content. Therefore many use mint leaves as a mixture of herbal drinks [20].

CONCLUSION

Based on the results of the analysis, the antioxidant activity of the combination of *Centella asiatica* leaf extract and mint leaf extract was included in the strong category with IC₅₀ results between 50-100 ppm.

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