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Antioxidant Activity of Hydroalcoholic Extract of Puguntano Herbs and Andaliman Fruits by Cuprac Methods

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ABSTRACT

Free radical damage to the body can be prevented and repaired using antioxidants. Various natural ingredients native to Indonesia contain antioxidants, which are needed to improve people's health at an affordable cost. Puguntano (*Picria fel-terrae*) and Andaliman (*Zanthoxylum acanthopodium* DC.) are natural ingredients that can be used as antioxidants. This research determined the antioxidant activity and differences in IC_{50} values of hydroalcoholic extracts from Puguntano (*Picria fel-terrae* Lour) herbs and Andaliman (*Zanthoxylum acanthopodium* Fruits). Hydroalcoholic extracts were prepared by maceration using 90%, 80%, 70%, 60% and 50% ethanol. Antioxidant capacity was measured using the CUPRAC method, and quercetin was used as a positive control. The results were showed that the IC_{50} value for hydroalcoholic extracts of Puguntano herbs (27.02 ± 0.15 µg/mL, 25.94 ± 0.22 µg/mL, 25.12 ±0.15 µg/mL, 22.07 ± 0.23 µg/mL, and 23.86 ±0.18 µg/mL) and for Andaliman fruits (41.65 ± 0.30 µg/mL, 36.59 ± 0.40 µg/mL, 34.13 ± 0.44 µg/mL, 25.46 ± 0.30 µg/mL, 25.39 ± 0.30 µg/mL) respectively and quercetin 2.21 ± 0.02 µg/mL. Hydroalcoholic extracts of Puguntano herbs and Andaliman fruits have antioxidant activity, with each concentration having an IC_{50} of less than 50 µg/mL. The test sample's antioxidant activity is indicated by its lower IC_{50} value.

Keywords: Antioxidant; Hydroalcoholic extract; IC_{50}; *Picria fel-terrae* Lour; *Zanthoxylum acanthopodium* DC Fruits

INTRODUCTION

Free radicals pose a threat to human health since they can result in degenerative conditions like diabetes, cancer, atherosclerosis, and high blood pressure. Free radicals are reactive molecules; this is because these molecules have one or more electrons to restore balance. Free radicals will try to obtain electrons from other molecules or remove unpaired electrons (Di Meo & Venditti, 2020; Ionita, 2021). One way to prevent the formation of free radicals is by increasing antioxidants (Bratovcic, 2020; Pisoschi et al., 2021). Antioxidants can supplement free radicals' lack of electrons and inhibit free radical formation's chain reaction (Martemucci et al., 2022). Finding sources of antioxidants from natural ingredients in Indonesia is needed to improve the quality of public health at relatively affordable costs.

Puguntano (*Picria fel-terrae*) and Andaliman (*Zanthoxylum acanthopodium* DC.) are natural ingredients that can be used as natural antioxidants. Puguntano from Tiga Lingga Village, Dairi Regency, North Sumatra Province, is often used for various diseases such as rheumatism, gout and diabetes (Satria et al., 2019a). Several studies show the use of puguntano as an anti-inflammatory, anthelmintic, antidiabetic, diuretic effect, cardioprotective effect, anti-breast cancer and inhibiting muscarinic receptor-3 and immunomodulator (Auliafendri et al., 2019a; Auliafendri et al., 2019b; Dalimunthe et al., 2023; Dalimunthe et al., 2024).

Andaliman is a plant found growing wild in the Tapanuli area and used as a spice. Andaliman has the speciality of typical Batak cuisine, which generally has a longer shelf life. Indian people have used Andaliman to treat leprosy and skin diseases such as abscesses and paralysis (Satria et al., 2019b; Ompusunggu & Irawati, 2021). Several studies state that Andaliman contains many compounds such as phenol hydroquinone, flavonoids, steroids/triterpenoids, tannins, glycosides, essential oils, alkaloids, coumarins, lignans, amides, and terpenes (Anjelisa et al., 2019;

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Satria et al., 2019c; Satria et al., 2023). Andaliman fruit extract has anticancer activity and cardioprotective effects (Syari et al., 2019; Angela et al., 2023).

This research was conducted to test the antioxidant activity of the hydroalcoholic extract of the Puguntano herbs and Andaliman fruit and determine the difference in antioxidant IC_{50} values using the CUPRAC (*Cupric Ion Reducing Antioxidant Capacity*) method. The advantages of the CUPRAC method are that the CUPRAC reagent is quite selective because it has a low reduction potential value, is fast, is a more stable reagent, can be obtained from other reagents, can be used for hydrophilic or lipophilic antioxidants at physiological pH. In addition, this method is easy, simple, reliable, requires low costs, and can be used in conventional laboratories with simple standard equipment (Özyürek et al., 2011; Munteanu & Apetrei, 2021; Csicsor et al., 2023).

MATERIALS AND METHODS

Materials

The tools used in this research include aluminum foil, a stirring rod, Glass Beaker (Iwaki), a porcelain cup, a chamber, a 250 mL separating funnel (pyrex), desiccator, Erlenmeyer (pyrex), measuring cup (Pyrex), plastic cuvette, micropipette (Eppendorf), tweezers, dropper pipette, rotary evaporator (Heidolph), sonicator, spatula, ultraviolet-visible spectrophotometer (Shimadzu), analytical balance (Mettler toledo), vial, vortex and maceration container. Meanwhile, the materials used in this research were Andaliman fruit and Puguntano herbs. The materials used for extracts and antioxidant measurements were ethanol, distilled water, ammonium acetate buffer, Neocuproin (Sigma), $CuCl_{2.2}H_2O$, and Quercetin (Muhammad et al., 2023).

Extract Preparation

The extraction process is carried out by maceration. To prepare the tools and materials, weigh 50 grams of Puguntano herb powder and put it in a round-bottomed flask. Then, the hydroalcoholic solvent with absolute ethanol-water was added with varying concentrations of 90%, 80%, 70%, 60%, 50%, and then the maceration process was carried out. The maceration process was carried out for seven days with stirring every day. They are filtering using a Buchner funnel. The filtrate is collected while the powder dregs are remaciated. The collected filtrate is concentrated using a rotary evaporator at a temperature of 50°C to remove the solvent until a thick extract is obtained (Nazliniwaty et al., 2021).

Test of Antioxidant Activity

Various methods can be used to measure antioxidants in samples, but only one method is considered the most ideal. Several methods that can be used to measure antioxidant activity include DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2.2-azinobis (3-ethyl benzothiazoline-6-sulfonate)) and FRAP (Ferric Reducing Antioxidant Power). These three methods use the same principle: the ability of antioxidant compounds to reduce free radicals or oxidants. The difference is in the free radical compounds used, namely ABTS and DPPH, while FRAP tests the ability of antioxidant compounds to reduce ferric, which is an oxidation catalyst (oxidizer). In practice, these tests are divided based on their mechanism of action, namely electron transfer and hydrogen atom transfer (Pande & Chanda, 2020; Xiao et al., 2020). Methods based on hydrogen atom transfer (HAT) are used to measure antioxidant activity by capturing free radicals through hydrogen atom donation. Some examples of these methods include ORAC, TRAP, β-Carotene bleaching assay, and others (Spiegel, 2022). Electron transfer (ET) based methods are used to measure the ability of antioxidants to reduce free radicals through electron transfer. Some examples of these methods include FRAP, CUPRAC, FIC, DMPD, and others (Ivanova et al., 2020). The DPPH and ABTS methods use both bases, HAT and ET. Various activity measurement methods can produce diverse antioxidant mechanisms of action (Parcheta et al., 2021; Capanoglu et al., 2021). The influence of the chemical structure of antioxidants, sources of free radicals, and the physicochemical properties of different sample preparations cause this. Therefore, selecting an appropriate and selective method for analyzing antioxidant activity for a particular type of sample is necessary.

The CUPRAC method was utilized in this work to determine antioxidant activity since the CUPRAC reagent is a selective reagent with a low reduction potential value. The CUPRAC reagent oxidizes antioxidant thiols relatively quickly, and because it is redox, it is also a selective reagent. These are the advantages of this method over other antioxidant measurement techniques. More stable and readily available than other chromogenic reagents are CUPRAC reagents (Munteanu & Apetrei, 2021). The antioxidant activity value, represented by the IC_{50} (Inhibitory Concentration) value, is ascertained by the UV-Vis

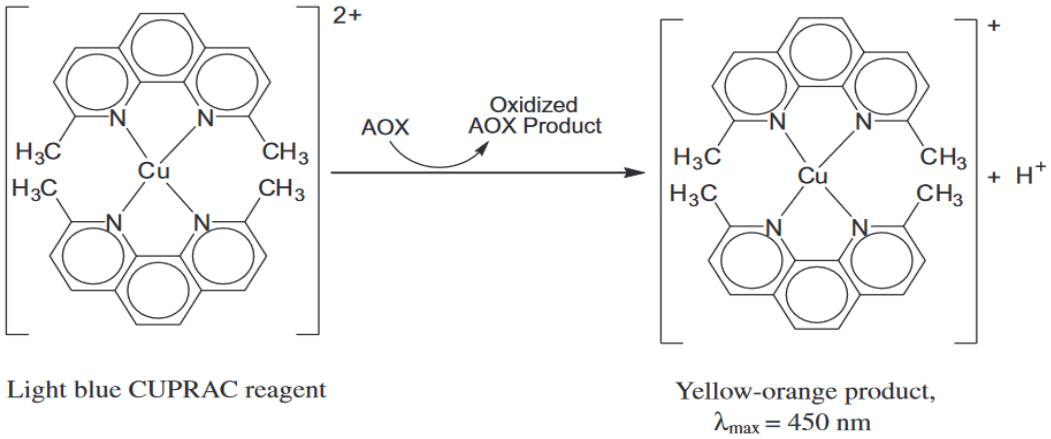

Figure 1. The CUPRAC reaction and chromophore: bis(neocuproine)copper(I) chelate cation

Table I. IC_{50} values of test samples and quercetin comparison standards

Sample		IC_{50} (µg/mL)
Quercetin		2.21 ± 0.02
Hydroalcoholic extracts of Puguntano herbs	90%	27.02 ±0.15
	80%	25.94 ±0.22
	70%	25.12 ±0.15
	60%	22.07 ±0.23
	50 %	23.86 ±0.18
Hydroalcoholic extracts of Andaliman fruits	90%	41.65 ±0.30
	80%	36.59 ±0.40
	70%	34.13 ±0.44
	60%	25.46 ±0.30
	50 %	25.39 ±0.30

spectrophotometer. This number indicates the concentration of the test substance, which has a 50% capacity to trap free radicals (Muhammad et al., 2023).

RESULT

We tested each plant to see the antioxidant activity based on the value IC_{50} with the CUPRAC method. The IC_{50} value, derived using a regression equation from the relationship between the concentration of the material under test and its percentage reducer, expresses the findings of assessing antioxidant activity. The IC_{50} results are displayed in Table I.

The results in Table I show that the hydroalcoholic extract of Puguntano herbs and Andaliman fruit with various solvents has potent antioxidant activity. According to the Blois classification, the level of antioxidant power of a compound is said to have potential antioxidant activity if the IC_{50} value is <50 µg/mL, a potent antioxidant if the IC_{50} value is between 50-100 µg/mL, a moderate antioxidant if the IC_{50} value is 101-150 µg/mL, and weak antioxidant if the IC_{50} value is between 150-200 µg/mL (Flieger et al., 2021). However, compared with the benchmark quercetin, the antioxidant activity of both plants was lower. Quercetin has a high antioxidant activity because it is a pure compound. Quercetin is a flavonol from polyphenolic flavonoid compounds found in almost every type of plant, and standard quercetin is a natural antioxidant with vigorous antioxidant activity (Batiha et al., 2020).

DISCUSSIONS

These two plants are generally local in North Sumatra, especially in the Batak ethnic. Puguntano is often used for diarrhoea, pain relief, increasing body endurance, and even as an anti-ageing agent (Harfina et al., 2012). Meanwhile, Andaliman fruit is often used as a spice in Batak cuisine and as an ingredient to cure coughs and make traditional health drinks (Silalahi et al., 2015).

The results of this study showed that the lowest IC_{50} value was 60% for Puguntano herbs and 50% for Andaliman fruit. The smaller the Solvent concentration, the stronger the

antioxidant. Most phenolic compounds, especially glycosides, are more soluble in polar solvents like water (Khaerunnisa et al., 2022). So, the solubility of compounds occurs more in solvents with a relatively large percentage of water. Flavonoids generally dissolve more easily in water or polar solvents because they bind to sugar groups (Kumalasari et al., 2023). The antioxidant activity of the flavonoid compounds contained in Puguntano and Andaliman is thought to be the cause of these two samples having potent antioxidants (Lubis et al., 2019; Satria et al., 2019b; Satria et al., 2019c; Satria et al., 2023). Flavonoids can chelate metals in glucosides (which contain glucose side chains) or inaccessible forms called aglycones, which transfer electrons or hydrogen atoms and act as an antioxidant (Vuolo et al., 2019). The principle of the CUPRAC method is based on a simple oxidation-reduction reaction between antioxidants and free radicals, which can be measured by reducing cupric ions (Cu^{2+}) to cupric ($Cu^{+)}$ through electron donation by antioxidants. Cu(II)-neocuproine (Cu^{2+}-$(Nc)_2$) reagent is used in this procedure as a chelating and oxidizing agent. A change in colour from yellow to orange indicates the presence of antioxidant activity. At 450 nm, the results of the Cu^{2+} ion reduction process can be measured. Copper will be decreased, and antioxidants will be oxidized. Bis (Neocuproin) copper (II) ($Cu\ (Nc)_2\ ^{2+}$) is the chromogenic reagent used in the CUPRAC technique (Figure 1) (Özyürek et al., 2011; Gulcin, 2020).

The high IC_{50} value of the extract is also influenced by the solution used in the extraction process. In this research, the solution used was a polar solvent. Polar solvents have a solid ability to enter plant cells. Apart from that, the bioactive components contained in simplicia outside the cells are easily dissolved by polar solvents. Thus, hydroalcoholic extracts contain more phenolic and flavonoid components than non-polar solvents (Ezez & Tefera, 2021; Saha et al., 2021). This causes the bioactive levels in Puguntano herbal extracts and Andaliman fruit with hydroalcoholic solvents to be higher, so their antioxidant activity is very high. Phenolic compounds are known to have antioxidant activity. Phenolic compounds are secondary metabolites that maintain the health of the human body.

The presence of chemical compounds in plants, such as phenols, flavonoids and tannins, indicates the possibility of antioxidant activity, and this antioxidant activity can help prevent disease through free radical scavenging activity (Swallah et al., 2020; Khiya et al., 2021; Yu et al., 2021).

CONCLUSION

Based on the IC_{50} value carried out using the CUPRAC method, it shows that each concentration of hydroalcoholic extract from Puguntano herbs (*Picria fel-terrae* Lour) and Andaliman fruit (*Zanthoxylum acanthopodium* DC. Fruits) has an IC_{50} of no more than 50 µg/mL so that it has antioxidant activity in the very high category strong.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in conducting this study.

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