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Evaluation of Antioxidant Activity in Traditional Indonesian Herbal Medicine (Jamu) Using ABTS and DPPH Testing Methods

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ABSTRACT
Herbal medicines are widely consumed as natural antioxidants to prevent oxidative stress-related diseases. Accurate assessment of antioxidant activity is essential, yet different methods may yield varying results. This study contributed to compare the antioxidant activities of ten commercial herbal medicines using ABTS (2,2'-azinobis- (3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (2,2-diphenyl-1picrylhydrazyl) assays and evaluate the correlation between both methods. Ten herbal formulations were collected from local producers in Samarinda, Indonesia. Each product was mixed with low-fat milk (1:10 w/v) to enhance bio-accessibility and extracted via sonication and centrifugation. Antioxidant activity was analyzed using ABTS and DPPH radical scavenging assays. Percentage inhibition and IC_{50} values were calculated. Statistical analysis was performed using GraphPad Prism 9.5.0 with Pearson correlation at a 95% confidence level. ABTS inhibition ranged from 10.54% to 72.73%, while DPPH ranged from 8.89% to 49.03%. IC_{50} values were lower in ABTS (13.51–105.36 µg/mL) than in DPPH (20.11–165.50 µg/mL). A moderate positive correlation (r=0.5390) between inhibition results was observed but was not statistically significant (p=0.1079). Among all samples, the herbal formulation containing turmeric, betel leaf, areca nut, and manjakani exhibited the highest antioxidant activity with 72.73% inhibition (ABTS) and an IC_{50} of 13.51 µg/mL. The differing sensitivities of ABTS and DPPH assays suggest that both methods should be used complementarily to obtain a comprehensive antioxidant profile of herbal products.

KEYWORDS
ABTS; Antioxidant activity; DPPH; Herbal medicine; Jamu

1. INTRODUCTION

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation of the spread of oxidation chain reactions [1], [2], [3]. Generally, there are two basic categories of antioxidants: natural and synthetic. Natural antioxidants are found in plants, animals, and even microorganisms, and include familiar compounds like flavonoids, polyphenols, and vitamins C and E. On the other hand, synthetic antioxidants such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) are produced chemically and are often added to food products to slow down spoilage and extend shelf life [4], [5]. Recently, interest in the discovery has increased rapidly; natural antioxidants are used in food or medicinal ingredients to replace synthetic antioxidants, which are limited due to their carcinogenicity [6].

Several in vitro methods have been developed to evaluate antioxidant activity, include the ABTS, DPPH, and FRAP (Ferric Reducing Antioxidant Power) assay. While all three are widely applied, each differs in reaction mechanism and sensitivity. The ABTS and DPPH assays measure the ability of

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antioxidants to scavenge free radicals via electron or hydrogen atom transfer, whereas the FRAP assay evaluates reducing power based on the reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions under acidic conditions. These mechanistic differences can influence the interpretation of antioxidant activity, particularly in complex matrices such as herbal formulations. The ABTS and DPPH methods were selected in this study due to their broad applicability to both hydrophilic and lipophilic antioxidant compounds in food and herbal products [7].

Jamu is a traditional Indonesian drink made from natural ingredients such as spices, medicinal plants, roots, leaves, and fruits [8], [9], [10]. Jamu has been used for generations as an herbal medicine to maintain health, increase endurance, and treat various diseases [11], [12]. Common ingredients often used in making jamu include turmeric, ginger, java turmeric, aromatic ginger, and tamarind. Herbal medicine made with a combination of phyllanthus (*Phyllanthus niruri*), turmeric, jungrahap (*Curculigo orchioides*), liquorice (*Glycyrrhiza glabra*), and binahong (*Anredera cordifolia*) spices has an antioxidant content of ABTS IC_{50} 27.17 µg/mL and DPPH 28.37 µg/mL [13]. The second variant combines bangle (*Zingiber purpureum*), bitter ginger (*Zingiber zerumbet*), tamarind, *manjakani* (*Quercus infectoria*), and turmeric has an ABTS IC_{50} 26.16 µg/mL and DPPH 29.56 µg/mL. These traditional herbal components are known to contain phenolic compounds, flavonoids, and other phytochemicals with high antioxidant potential. The high antioxidant content in herbal medicine causes an increase in consumption every year, and no less than 80% of people worldwide rely on these products for some parts of primary health care [14]. For example, in countries like China and India, traditional herbal medicines are not only culturally significant but also formally integrated into public healthcare systems, where they are used alongside modern treatments [15], [16], [17]. In Indonesia, everyday herbal drinks such as jamu *kunyit asam* and jamu *temulawak* remain popular choices among the public for maintaining health and boosting antioxidant defenses [18].

The choice of antioxidant activity measurement methods, such as ABTS and DPPH, dramatically affects the results and interpretation of antioxidant activity in samples. Each method has a different working principle, sensitivity, and application, which can provide varying information about the antioxidant capacity of a material [19]. ABTS measures the ability of antioxidants to reduce blue/greenish ABTS+ cation radicals to colorless forms used to calculate the antioxidant activity of hydrophobic (fat-soluble) and hydrophilic (water-soluble) compounds. In contrast, DPPH measures the ability of antioxidants to donate electrons or hydrogen to reduce free radicals. DPPH is purple to a colorless or pale-yellow form and is very sensitive to lipophilic (fat-soluble) antioxidants [20]. So, this research contributed to compare the antioxidant activity content of the ABTS and DPPH methods and to evaluate their relationship to various types of commercial herbal medicines.

2. MATERIALS AND METHODS

2.1. Materials

The materials used in this study were aquadest is produced at the Faculty of Agriculture, Mulawarman University, ethanol absolute (Merck), DPPH from Sigma Aldrich (USA), ABTS from Sigma Aldrich, potassium persulfate (Merck), and low-fat milk (Tropicana Slim) from supermarket in Samarinda.

2.2. Sample Preparation

Samples of herbal medicine were taken from UMKM (micro, small, and medium enterprises) in Samarinda city. The types of herbal medicine and their compositions are presented in Table 1. The consumption serving of each herbal medicine sample was blended with low-fat milk in a 1:10 (w/v) ratio to improve its bio-accessibility [21]. The mixture was then treated with ultrasound using a Delta D68H sonicator for 10 minutes and gently stirred to achieve an even consistency. After this process, the samples were centrifuged at 1500 RPM for 30 minutes using an Oregon LC-04S centrifuge to separate the liquid supernatant from any remaining solid residue. The clear supernatant was collected and used for the antioxidant activity analysis.

Table 1. Types of herbal medicine and their compositions.

Types of herbal medicine	Composition
Turmeric tamarind	Turmeric, ginger, tamarind, water, palm sugar, granulated sugar
Turmeric galangal	Turmeric, roasted rice, galangal, palm sugar, water
Turmeric, betel, areca nut, *manjakani*	Turmeric, betel, young areca nut, *manjakani*, ginger, tamarind, water, palm sugar, granulated sugar
Turmeric betel	Turmeric, betel, ginger, tamarind, palm sugar water, granulated sugar
Turmeric ginger	Turmeric, ginger, red ginger, granulated sugar, palm sugar, water
Curcuma	Java turmeric, tamarind, palm sugar, granulated sugar, water
Black turmeric	Black turmeric, tamarind, palm sugar water, granulated sugar
Fingerroot	Fingerroot, tamarind, palm sugar water, granulated sugar
White turmeric	White turmeric, palm sugar, granulated sugar, water, tamarind
Tembora	*Tembora* leaves, turmeric, aromatic ginger, red ginger, palm sugar, white sugar, water, tamarind

2.3. Antioxidant Activity Evaluation Using ABTS Method

The ABTS stock solution was prepared by reacting the ABTS solution (7 mM) with the 2.45 mM potassium persulfate solution in equal amounts. The mixture was allowed to stand in the dark condition at room temperature for 12–16 hours before use. The ABTS working solution was obtained by diluting the stock solution in ethanol to produce an absorbance of 0.70 at 734 nm. Then, 2.0 mL of ABTS solution was mixed with 1 mL of extract at various concentrations (0.5–5.0 mg/mL). The mixture was then incubated at room temperature for 10 minutes in the dark mode room. Controls were prepared by mixing 1 mL of ABTS solution with 2 mL of distilled water. Absorbance was measured against a blank at 734 nm using a spectrophotometer (Eppendorf Bio Spectrometer® basic) [22]. Samples were prepared and measured in triplicate. The percentage of scavenging activity of each extract on ABTS was calculated as % inhibition using the equation (1).

$$\%\ inhibition = \frac{(control\ absorbance - \ sample\ absorbance)}{control\ absorbance} \times 100 \quad (1)$$

2.4. Antioxidant Activity Evaluation Using DPPH Method

Total antioxidants were carried out using the spectrophotometric method (Eppendorf Bio Spectrometer® basic) with DPPH [7]. A total of 1 mL extract that had been diluted in ethanol was added to 1 mL of DPPH (0.15 mM in ethanol). At the same time, a control consisting of 1 mL of DPPH with 1 mL of ethanol was prepared. The reaction mixture was mixed well by hand and then incubated in the dark condition at room temperature for 30 minutes. The absorbance was measured at 515 nm. The ethanol was used as a blank. The DPPH capability of the extract was calculated using the equation (1).

Parameters for interpreting ABTS and DPPH test results involve calculating the IC_{50} value. IC_{50} is the concentration of sample solution required to reduce antioxidant activity by 50%. A smaller IC_{50} value indicates a higher antioxidant content. The IC_{50} value is obtained from a linear equation that relates the percentage of DPPH radical inhibition to various sample concentrations.

2.5. Data Analysis

The results are expressed as three measurements' mean and standard deviation (SD). Statistical analysis was performed using the student's t-test, and $P<0.05$ was considered significant. The correlation between the data obtained was calculated using the correlation coefficient statistical option of the GraphPad Prism Version 9.5.0 device.

3. RESULTS AND DISCUSSION

3.1. Percentage of Free Radical Inhibition of Commercial Herbal Medicine Using DPPH and ABTS Methods

The effectiveness of antioxidant compounds in a percentage concentration of 20 mg/mL is presented in Table 2. The percentage of free radical inhibition in herbal medicine using the ABTS method ranged from 10.54% to 72.73%, while the DPPH method ranged from 8.89% to 49.03%. A higher inhibition percentage reflects a stronger ability of the sample to neutralize free radicals [23]. Among the tested products, the highest antioxidant activity was observed in turmeric betel areca nut *manjakani* (72.73%), turmeric betel (57.53%), and turmeric tamarind (61.34%) in the ABTS assay, all showing inhibition above 50%. Conversely, the lowest ABTS values were recorded for black turmeric (11.76%) and curcuma (10.54%). The DPPH method generally produced lower inhibition percentages, with only one sample, turmeric betel, exceeding 50% (51.99%). These differences in inhibition values between the ABTS and DPPH assays reflect the distinct chemical properties of each method. ABTS radicals are reactive with both hydrophilic and lipophilic antioxidant compounds, allowing broader detection of antioxidant activity. In contrast, DPPH radicals are more selective and primarily react with lipophilic antioxidants, which can result in lower sensitivity to phenolic compounds and water-soluble antioxidants typically found in herbal medicines [19], [24].

For instance, the fingerroot sample showed a higher inhibition value in the DPPH method (49.03%) than in ABTS (34.62%), indicating the presence of lipophilic antioxidant compounds in the formulation. On the other hand, the ABTS assay more effectively detected the antioxidant activity of turmeric tamarind and turmeric betel areca, nut *manjakani*, likely due to the dominance of polyphenols and flavonoids with higher solubility in aqueous or mixed-phase systems [25], [26], [27]. This finding is consistent with a study by Niang et al. (2021) [28], which demonstrated that ABTS inhibition values were generally higher than DPPH values when applied to various plant extracts [29], [30], [31].

Table 2. Percentage of inhibition of ABTS and DPPH methods at a concentration of 20 mg/mL.

Sample	ABTS (%inhibition)	DPPH (%inhibition)
Turmeric tamarind	61.34 ± 0.57	28.13 ± 0.01
Turmeric galangal	37.52 ± 0.39	46.51 ± 0.48
Turmeric betel areca nut *manjakani*	72.73 ± 0.34	37.96 ± 0.39
Turmeric betel	57.53 ± 0.15	51.99 ± 0.23
Turmeric ginger	47.77 ± 0.77	32.23 ± 0.32
Curcuma	10.54 ± 0.30	35.38 ± 0.25
Black turmeric	11.76 ± 0.53	8.89 ± 0.12
Fingerroot	34.62 ± 1.27	49.03 ± 1.80
White turmeric	15.24 ± 0.82	17.57 ± 0.30
Tambora	15.83 ± 1.71	18.19 ± 0.60

3.2. Inhibitory Concentration 50 (IC_{50}) ABTS and DPPH methods of Commercial Herbal Medicine

The antioxidant capacity expressed in ABTS and DPPH in various commercial herbal medicines is presented in Table 3. Antioxidant activity was measured quantitatively using IC_{50}. IC_{50} is the concentration of the test solution that can reduce DPPH by 50%. Specifically, a compound is categorized as a powerful antioxidant if the IC_{50} value is less than 50 ppm, as a potent antioxidant if the IC_{50} value is between 50–100 ppm, as a moderate antioxidant if the IC_{50} value is between 100–150 ppm, and as a weak antioxidant if the IC_{50} value is more than 151 ppm [30].

The antioxidant activity value of IC_{50} for ABTS ranges from 13.1 µg/mL to 105.36 µg/mL, while the IC_{50} for DPPH values range from 20.11 µg/mL to 165.50 µg/mL. In turmeric-betel-areca nut-*manjakani* herbal medicine, the ABTS method effectively inhibited free radicals with an IC_{50} value of 13.51 µg/mL, while the DPPH method showed a much higher IC_{50} value of 25.51 µg/mL, indicating that turmeric-betel-areca nut-*manjakani* is more effective in counteracting ABTS-type free radicals than DPPH. This can be attributed to the chemical nature of antioxidant compounds present in the formulation,

such as curcumin and ascorbic acid, which are more reactive with $ABTS^+$ cation radicals that are soluble in both hydrophilic and lipophilic environments. In contrast, DPPH is more selective for lipophilic antioxidants, and may not interact as efficiently with the hydrophilic antioxidants in this sample [31], [32].

Table 3. IC_{50} antioxidant activity capacity of herbal medicine using ABTS and DPPH methods.

Sample	ABTS (IC_{50})	DPPH (C_{50})
Turmeric tamarind	15.82 ± 0.17	26.82 ± 8.15
Turmeric galangal	28.78 ± 0.12	21.43 ± 0.45
Turmeric betel areca nut *manjakani*	13.51 ± 0.08	25.51 ± 0.17
Turmeric betel	17.55 ± 0.08	21.28 ± 0.05
Turmeric ginger	24.48 ± 0.44	37.61 ± 0.53
Curcuma	105.36 ± 8.93	26.17 ± 0.12
Black turmeric	103.31 ± 5.60	165.50 ± 8.86
Fingerroot	36.73 ± 0.67	20.11 ± 0.58
White turmeric	80.67 ± 8.21	87.21 ± 6.86
Tambora	93.99 ± 9.93	75.08 ± 6.33

A similar free radical scavenging ability with a higher affinity in the ABTS type method can be observed in white turmeric, black ginger, ginger turmeric, betel turmeric and betel pinang *manjakani* turmeric. The ABTS assay appears more effective for compounds that can interact in both hydrophilic and lipophilic environments, leading to lower IC_{50} values for these samples. Antioxidant compounds have specific chemical structures such as curcumin compounds in turmeric can form stable resonance structures after donating electrons or hydrogen, which allows them to easily donate electrons or hydrogen to ABTS free radicals, thus neutralizing the activity of these free radicals [33], [34]. The mechanism of formation and neutralization of ABTS free radicals by curcumin is that ABTS is radicalized into its free radical form, using potassium persulfate as an oxidizing agent: $ABTS + K_2S_2O_8 \rightarrow ABTS + +2K^+ \rightarrow$ and neutralization with Curcumin ($C_{21}H_{20}O_6$) become $ABTS^+ + C_1H_0O_6 \rightarrow ABTS + C_1H_9O_6^+$ [35], [36], [37].

On the other hand, for samples such as turmeric aromatic ginger, curcuma, fingerroot, and tambora, the DPPH method showed lower IC_{50} values, indicating higher efficiency in neutralizing DPPH-type free radicals. This suggests that these samples may contain a higher concentration of lipophilic antioxidants that interact more readily with DPPH radicals, which are known to be more selective toward nonpolar compounds [38], [39]. Overall, this comparison shows that the ABTS method tends to give lower IC_{50} values than the DPPH method, indicating that the ABTS method may be more sensitive in measuring antioxidant activity. The differences between ABTS and DPPH highlight the need for using multiple assays to obtain a more comprehensive understanding of antioxidant capacity, as different methods react differently with various types of antioxidant compound.

3.3. Comparison and Correlation of Antioxidant Activity of ABTS and DPPH Methods on Commercial Herbal Products

This study evaluated the antioxidant activity of various commercial herbal products using two standard test methods, namely DPPH and ABTS. The measurement results were then compared to assess the suitability and consistency between methods. Figure 1A displays the relationship between the percentage inhibition values of DPPH and ABTS of ten herbal samples at a concentration of 20 µg/mL. Visually, the distribution of data points on the graph shows a tendency for a positive relationship, but not too strong. This is also reflected in the Pearson correlation coefficient (r) value of 0.5390, which indicates a moderate positive relationship. However, the p-value of 0.1079 indicates that this correlation is not statistically significant (α=0.05) so a linear relationship between the two inhibition methods cannot be concluded with certainty. This discrepancy is seen, for example, in the fingerroot sample, which showed a high inhibition value in the DPPH method (49.03%) but only moderate in ABTS (34.62%).

In contrast, Turmeric betel areca nut showed the highest inhibition in ABTS (72.73%) but only 37.96% in DPPH. This shows that the two methods do not always provide equivalent results for certain

types of antioxidants in herbal products [40]. Figure 1B illustrates the relationship between the IC_{50} values of the DPPH and ABTS methods. The correlation between these two parameters is weaker than the inhibition percentage, as indicated by the Pearson r value of 0.4095, with an extensive 95% confidence interval (–0.2966 to 0.8261) and a p-value of 0.2399, which is also not statistically significant. This means that although there is a tendency for herbs with low IC_{50} in ABTS to also have low IC_{50} in DPPH (and vice versa), this pattern is not consistent. This is evident in some samples, such as Curcuma, which has a very high IC_{50} in ABTS (105.36 µg/mL) but much lower in DPPH (26.17 µg/mL). Similarly, black turmeric showed the highest IC_{50} in both methods. However, the difference was huge (103.31 µg/mL in ABTS and 165.50 µg/mL in DPPH), indicating different responses to the types of radicals from each method. The difference in results between the two methods can be explained by the nature of the chemical reactions underlying the ABTS and DPPH methods. The DPPH method is generally more sensitive to lipophilic antioxidant compounds, while ABTS is able to react with both hydrophobic and hydrophilic compounds [19], [20], [41]. Therefore, discrepancies in results may occur depending on the composition of active compounds in each herbal product. This variation shows the importance of using more than one method in evaluating antioxidant activity, especially in complex materials such as herbal products. This variation shows the importance of using more than one method in evaluating antioxidant activity, especially in complex materials such as herbal products.

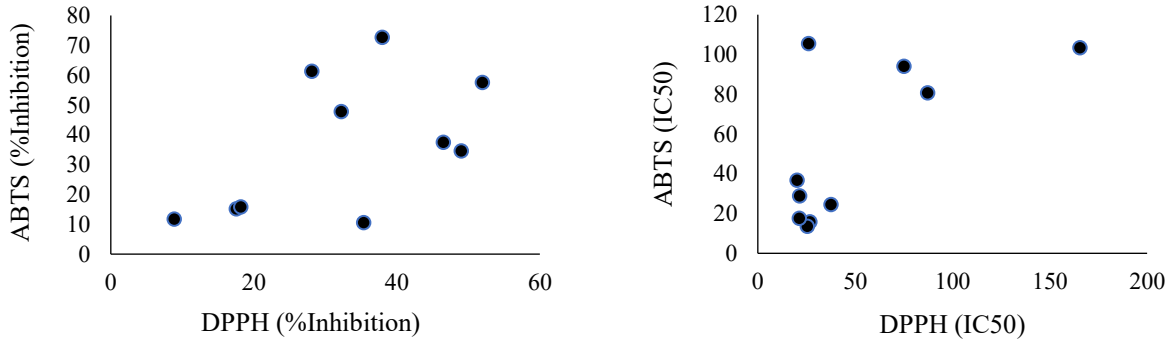

Figure 1. Relationship between ABTS and DPPH methods in commercial herbal medicines, showing (A) percentage inhibition of free radicals and (B) IC_{50} values.

4. CONCLUSION

This study compared the antioxidant activity of various commercial herbal medicines using the ABTS and DPPH methods. The results showed that the ABTS method was more sensitive to antioxidant activity than DPPH. Herbal medicines with turmeric betel nut *manjakani*, turmeric betel, and turmeric tamarind showed high antioxidant effectiveness in the ABTS method. The correlation between the two methods showed a moderate positive correlation but was not statistically significant.

AUTHOR CONTRIBUTION

All authors contributed equally as the main contributors to this paper. All authors have read and approved the final version of the manuscript. **Rimbawan Apriadi**: Writing the original draft, reviewing, editing, and conducting formal analysis. **Kartika Sari**: Reviewing and editing the manuscript, as well as data curation and methodology. **Maulida Racmawati**: Investigating, providing resources, and reviewing the manuscript. **Muhammad Rafii Nur Fauzan**: Data collection, investigation, and manuscript review. **Miftakhur Rohmah**: Supervision and conceptualization of the study, managed the project, contributed to manuscript review and editing, and was responsible for securing funding.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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