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RESEARCH PAPER

Effect of different solvents on antioxidant activity of *Euphorbia hirta* L. extract

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Abstract: Euphorbia hirta L. is a species of plant renowned for its antioxidant properties. However, the optimal solvent for extracting these bioactive compounds to maximize antioxidant activity had not been systematically identified and compared. Previous knowledge suggested that solvent choice affects yield and efficacy; however, there was a lack of conclusive, comparative data specifically for E. hirta extracts using different solvents. The focus of this work was to investigate how different solvents affected the content of phenolics and flavonoids, as well as the antioxidant effect of E. hirta extract. The aerial part of E. hirta powder was extracted with ethanol, ethyl acetate, and n-hexane to obtain the crude extracts. Folin-Ciocalteu reagent and AlCl3 solution were used to assess the total phenolic and flavonoid contents, respectively. The ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) techniques were used to assess antioxidant activity. The total content of phenolics and flavonoids of E. hirta ethanol extract (127.827 ± 2.609 mg GAE/g extract and 28.507 ± 0.464 mg QE/g extract, respectively) was substantially higher than that of the other extracts ($p<0.0001$). The findings of the FRAP test exhibited that the E. hirta ethanol extract (48.009 ± 1.873 mg AAE/g extract) had a substantially greater reducing power value ($p<0.0001$) in comparison to other extracts. The ethanol extract of E. hirta had a considerably lower IC50 value (6.154 ± 0.063 ppm and 9.429 ± 0.183 ppm for DPPH and ABTS assays, respectively, with $p<0.0001$) than the other extracts. This study found that solvent polarity had a substantial influence on the antioxidant activity of E. hirta extracts, with ethanol being an optimal solvent for the extraction procedure.

Keywords: Antioxidant, *Euphorbia hirta*, Flavonoids, Phenolics, Solvent

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INTRODUCTION

Oxidative stress can be viewed as a disparity between the creation and storage of reactive substances (free radicals) in bodily tissues and cells, and the biological mechanism's ability to eliminate these harmful molecules [1]. A link has been found between oxidative stress and many diseases, including cardiovascular diseases, cancer, diabetes mellitus, neurological disorders, mental diseases, renal disease, lung disease, and ageing [2]. If the body's antioxidants are ineffective in overcoming free radicals, exogenous antioxidants are required.

Plants are an abundant source of antioxidants in nature because they contain a wide range of secondary metabolites. One of the plants that has good antioxidant activity is *Euphorbia hirta* L. This weed has long been utilized as traditional medicine in Indonesia. Certain health problems, including gastrointestinal disorders, skin conditions, and asthma,

have been successfully treated using this plant [3]. According to scientific research, *E. hirta* extract exhibits antioxidant, anticancer, anti-inflammatory, antidiabetic, and antibacterial effects [4–6]. The antioxidant action of *E. hirta* extract is mainly caused by the abundance of phenolic, flavonoid, and terpenoid components [7–9]. However, its content is primarily reliant on the solvent polarity, which ultimately influences its antioxidant activity. These classes of molecules have varying levels of polarity; therefore, the amount of phytochemicals which can be extracted relies on the solvent's degree of polarity throughout the extraction procedure [10]. In nature, phenolic compounds are frequently attached to sugar as glycosides, making them easily soluble in polar solvents. Still, flavonoids can be soluble in both polar solvents (in the form of glycosides) and semipolar solvents [11]. Steroids and terpenoids dissolve in nonpolar liquids.

Several prior studies have detected tannins and saponins in ethyl acetate extract. Semipolar solvents can be used to extract the molecule because the resonance in the benzene ring reduces its polarity. Steroids and terpenoids are frequently nonpolar compounds. Several steroid and terpenoid compounds include hydroxyl groups, which enable them to construct hydrogen bonds with ethyl acetate. Therefore, ethyl acetate can be applied to extract these chemicals [10]. Extraction of *E. hirta* can be carried out with solvents of different polarities to obtain extracts with optimal antioxidant effects.

Numerous studies have reported the antioxidant effects of E. hirta extract. Those studies conclusively demonstrated that *E. hirta* is a rich source of antioxidant compounds, validating its traditional use in herbal medicine. According to Basyal et al. [4], the *E. hirta* ethyl acetate extract contained 29.36 mg QE/g of total flavonoids and 288.10 mg GAE/g of total phenolics. Furthermore, the DPPH method analysis of the *E. hirta* ethyl acetate extract revealed that its antioxidant effect (IC_{50}) was 32.23 ppm. According to Tran et al. [6], the antioxidant effect (DPPH), total phenolic, and total flavonoid levels in the *E. hirta* methanol extract were 17.26±0.12 ppm, 18.92±1.33 mg QE/g, and 109.86±1.38 mg GAE/g, respectively. Although *E. hirta* has known antioxidant activity, direct comparisons of solvents with different polarities are limited. This study aimed to investigate the effect of solvent polarity levels on the total phenolics, total flavonoids, and antioxidant activity of E. hirta.

MATERIALS AND METHODS

Materials. The aerial parts of *E. hirta* were harvested in Badung Regency, Bali Province. The plant (Figure 1) was identified in the Pharmacognosy Laboratory, Faculty of Pharmacy, Universitas Gadjah Mada, under the identification number 3265/UN1/FA.2/BF/PT.01.06/2024. For the extraction process, the technical-grade ethanol, n-hexane, and ethyl acetate were utilized. 2,2-diphenyl-1-picrylhydrazyl, methanol, sodium hydroxide, folin-ciocalteu reagent, potassium acetate, aluminium chloride, sodium acetate, ferric chloride, acetic acid, and potassium persulfate, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid, gallic acid, quercetin, and 2,4,6-tris (2-piridil)-s-triazin were chemicals utilized for the assay.

Sample Preparation and Extraction. The principle of solvent polarity predominantly governs the efficiency of extracting any specific bioactive compound. Therefore, the strategic selection of solvents with varying polarities is not merely a methodological choice but a necessary prerequisite to comprehensively accessing and evaluating the plant's full medicinal potential. In the preparation step, the *E. hirta* aerial parts were cleaned under running water, drained, and heated in an oven (Memmert UN 75) at 50 °C for 3 days. The sample was then crushed into a powder (200 g) using a blender and macerated with three different solvents: ethanol (70% v/v), ethyl acetate, and n-hexane, for 24 hours. The sample solvent ratio used was 1:10. After the mixture was filtered, a rotary evaporator (IKA RV 8 V) was used to evaporate the filtrate until a thick, sticky extract was achieved.

Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC). TPC was determined by incubating a mixture of 1 mL of extract solution, 0.5 mL of Folin-Ciocalteu (7.5%), and 4 mL of sodium hydroxide solution (1% w/v in water) for 1 hour. A UV-Vis spectrophotometer (Genesys 10S) was used to measure the absorbance of the solution mixture at 730 nm. Gallic acid served as a reference. The findings were reported in milligrams of GAE/gram of extract. TFC was assessed by incubating a mixture of 0.5 mL of extract solution, 0.1 mL of aluminium chloride solution (10% w/v), 1.5 mL of ethanol, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water at 27°C for 30 minutes. Next, the solution's absorbance was detected at 415 nm. The results were depicted in milligrams of QE/gram of extract [12].

Antioxidant Assays. The FRAP test was performed using the technique described by Sekhon-Loodu & Rupasinghe [13] with modifications. FRAP reagent consists of TPTZ (10 mM), $FeCl_3$ (20 mM), and acetate buffer (300 mM, pH 3.6) with a ratio of 1:1:10 (v/v). The combination of 1 mL of extract solution and 2 mL of FRAP reagent was incubated for 15 minutes at 37 °C. The absorbance at 594 nm was measured. Ascorbic acid served as a reference. The total antioxidant capacity was reported in milligrams of AAE/gram of extract. The test for DPPH scavenging activity was conducted utilizing the approach outlined by Putra et al. [14]. The extract solution (2 mL) and DPPH reagent

(2 mL) mixture was incubated for 30 minutes in a dark environment, and its absorbance was recorded at 516 nm. Antioxidant activity was reported in the IC_{50} value. The test for $ABTS^+$ radical scavenging activity was conducted utilizing the procedure described by Lee et al. [15]. The $ABTS^+$ reagent consisted of 88 µl of potassium persulfate (140 mM), 5 ml of ABTS solution (7 mM), and methanol. The extract solution (1 mL) and $ABTS^+$ solution (2 mL) mixture was incubated for 6 minutes in a dark environment, and its absorbance was read at 516 nm. The mixture consisting of the extract and $ABTS^+$ reagent was incubated in a dark environment for six minutes. The absorbance was recorded at 747 nm. Antioxidant activity was reported in the IC_{50} value.

Figure 1. *Euphorbia hirta* L. plant

Data Analysis. To ensure the precision and reliability of the results, every procedure was conducted three times, and the mean ± standard deviation was used to display the results. To prepare the tables, Microsoft Excel 2019 was used. The difference between groups was statistically determined using one-way ANOVA followed by Tukey's post hoc test ($p < 0.0001$). GraphPad Prism 8 was utilized for statistical analysis.

RESULTS AND DISCUSSION

Extraction Yield. Extraction of *E. hirta* was carried out by maceration of 200 g of powdered simplicia with 2 L of ethanol (70% v/v), ethyl acetate, and n-hexane. Maceration was repeated 3 times. The extraction yield is depicted in Table 1. In comparison to n-hexane extract (2.4%) and ethyl acetate extract (3.9%), the *E. hirta* ethanol extract demonstrated a greater yield (21.5%). The quantity of secondary metabolites influences the extraction yield that the solvent can extract during the extraction process. When estimating the volume of extract generated during the extraction process, the yield value is crucial.

Table 1. Extraction yield

Extraction Solvent	Weight of simplicia (g)	Weight of extract (g)	Yield (%)
Ethanol (70% v/v)	200	42.9	27.2
Ethyl acetate	200	7.7	3.9
N-hexane	200	4.8	2.4

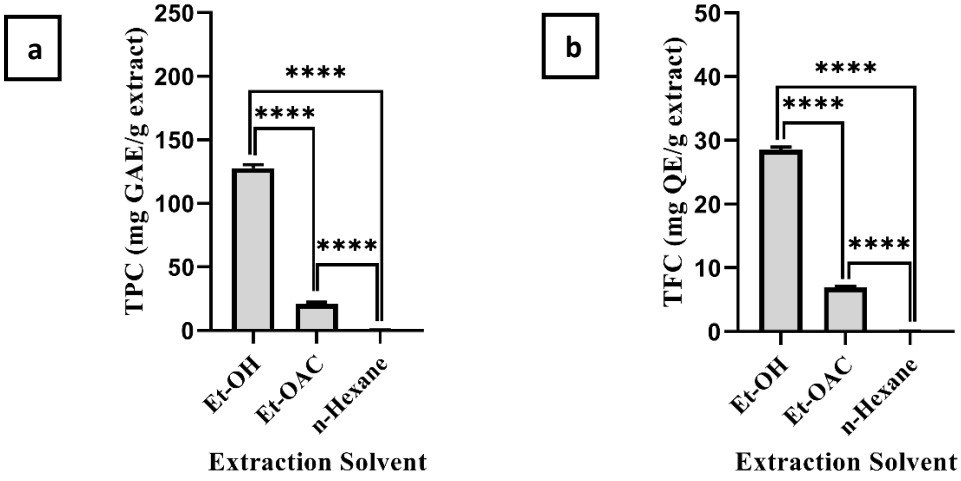

Figure 2. The TPC (a) and TFC (b) values of *E. hirta* extracts obtained with different solvents. Values are presented as mean ± SD ($n = 3$). Statistical significance was determined using one-way ANOVA followed by Tukey's test (**** $p < 0.0001$). Et-OH = ethanol, Et-OAc = ethyl acetate.

Additionally, yield data and secondary metabolites in a sample are highly correlated. The quantity of secondary metabolites increases with the yield [16]. In this work, the ethanol extract yielded the most significant amount compared to the other extracts (Table 1). It suggested that ethanol had a greater quantity of compounds that could be extracted than any other solvent. The researcher found that solvent

polarity has a significant impact on extraction yield. In polar solvents (methanol/ethanol), more secondary metabolites will be extracted than in semipolar solvents (ethyl acetate) and nonpolar solvents (acetone, chloroform, n-hexane) [17].

The TPC and TFC of *E. hirta* Extracts. As depicted in Figure 2, the TPC and TFC of the *E. hirta* ethanol extract are 127.827 ± 2.609 mg GAE/g extract and 28.507 ± 0.464 mg QE/g extract, respectively. These values are significantly higher than those shown by the ethyl acetate and n-hexane extracts ($p < 0.0001$). The TPC and TFC of the *E. hirta* ethyl acetate extract are 20.998 ± 1.317 mg GAE/g extract and 6.941 ± 0.209 mg QE/g extract, respectively. Meanwhile, the TPC and TFC of the *E. hirta* n-hexane extract were 0.427 ± 0.081 mg GAE/g extract and 0.074 ± 0.051 mg QE/g extract, respectively. Phenolics are one of the most significant types of phytochemicals found in herbs, acting as a protective agent against insects, parasites, and UV radiation [18]. Caffeic acid, ferulic acid, gallic acid, p-coumaric acid, and other phenolic acids are associated with various human health benefits, including antibacterial, antioxidant, antiproliferative, anti-inflammatory, and anticancer properties, which help individuals maintain their health [19]. Similarly, flavonoids, including flavanones, flavones, flavonols, anthocyanins, and isoflavonoids, are the most prevalent types of phenolic compounds, which are extensively dispersed in medicinal plants, fruits, vegetables, and cereals [20]. Additionally, these biomolecules exhibit numerous biological and pharmacological effects, featuring antioxidant, antimutagenic, anti-inflammatory, anticancer, antiallergic, and enzyme-regulating effects [21]. Several environmental conditions (water, air, soil, rainfall, temperature and altitude) and genetic changes across plant species or among individuals of the same species have a significant impact on the synthesis and amount of phenolics and flavonoids, as well as their pharmacological potential [22].

FRAP Assay. FRAP, or ferric reducing antioxidant power, has been the most routinely conducted antioxidant test. The capacity of the extract to reduce Fe^{3+} to Fe^{2+} is the basis for the FRAP assay. To preserve iron's solubility, the FRAP reaction is conducted at an acidic pH of 3.6. It lowers the ionization potential, which facilitates the transfer of hydrogen atoms, and raises the redox potential, which is the primary reaction mechanism. Fe^{3+} reduction to Fe^{2+} when TPTZ is present results in the formation of a complex with a blue colour [23]. In this work, a curve for calibration was made using ascorbic acid solution as a reference. **Table 2** shows the Fe^{3+} reducing power of *E. hirta* extracts. The findings demonstrated that the ethanol extract of *E. hirta* had a much greater reducing power than both the n-hexane and ethyl acetate extracts ($p < 0.0001$). The order of reducing power of *E. hirta* extracts is as follows: ethanol extract > ethyl acetate extract > n-hexane extract.

Table 2. Reducing the power of *E. hirta* extracts

Extraction Solvent	Reducing power (mg AAE/g extract)
Ethanol (70% v/v)	48.009 ± 1.873^{c}
Ethyl acetate	5.468 ± 0.048^{b}
N-hexane	0.000 ± 0.000^{a}

Values are presented as mean ± SD (n = 3). Different superscript letters (a–c) indicate significant differences based on ANOVA followed by Tukey's test ($p < 0.0001$).

DPPH Radical Scavenging Activity. The DPPH assay is a fundamental test that involves mixing plant extracts with a DPPH solution and measuring their absorption after a predetermined time interval [24]. The DPPH antioxidant test relies on DPPH's capacity to fade to purple, a steady free radical, simply because of the presence of antioxidants. When DPPH accepts electrons donated by antioxidants, DPPH undergoes a color fading, which can be measured quantitatively from the change in absorbance [25].

Table 3. DPPH radical scavenging activity of *E. hirta* extracts

Extraction Solvent	DPPH radical scavenging activity, IC_{50} (ppm)	Category
Ethanol (70% v/v)	9.429 ± 0.183^{a}	Very strong
Ethyl acetate	217.096 ± 4.335^{b}	Medium
N-hexane	1474.032 ± 12.122^{c}	Not active
Quercetin*	1.735 ± 0.032^{a}	Very strong

Values are expressed as mean ± SD (n = 3). Different superscript letters (a–c) indicate significant differences determined by ANOVA followed by Tukey's test ($p < 0.0001$). *Quercetin served as the positive control.

Table 3 shows the DPPH radical scavenging activity of *E. hirta* extracts. *E. hirta* ethanol extract showed extreme activity as indicated by its IC_{50} value of 9.429 ± 0.183 ppm. This value was much lower than those of ethyl acetate and n-hexane extracts ($p < 0.0001$). The ethyl acetate extract exhibited medium antioxidant activity with an IC_{50} of 217.096 ± 4.335 ppm. The n-hexane extract did not show antioxidant activity, as the IC_{50} value was very high (>500 ppm).

$ABTS^{+}$ Radical Scavenging Activity. The $ABTS^{+}$ radical scavenging technique is defined as an electron transfer-based method used to quantify the antioxidant effect of a compound by reducing the dark blue $ABTS^{+}$ radical cation to the colourless ABTS, which can be measured spectrophotometrically. **Table 4** below displays the findings of the $ABTS^{+}$ radical scavenging test. Ethanol and ethyl acetate extracts exhibited significant activity, as evidenced by IC_{50} values of

6.154 ± 0.063 ppm and 9.935 ± 0.155 ppm, respectively. However, the IC_{50} value of ethanol extract was much lower than those of ethyl acetate and n-hexane extracts ($p < 0.0001$). Meanwhile, n-hexane extract of *E. hirta* did not show antioxidant effect due to its very high IC_{50} value (> 500 ppm).

Table 4. $ABTS^+$ radical scavenging activity of *E. hirta* extracts

Extraction Solvent	$ABTS^+$ radical scavenging activity, IC_{50} (ppm)	Category
Ethanol (70% v/v)	6.154 ± 0.063[a]	Very strong
Ethyl acetate	9.935 ± 0.155[a]	Very strong
N-hexane	768.215 ± 16.554[b]	Not active
Quercetin*	0.776 ± 0.013[a]	Very strong

Values are expressed as mean ± SD ($n = 3$). Superscript letters (a and b) indicate significant differences based on ANOVA followed by Tukey's test ($p < 0.0001$). *Quercetin served as the positive control.

In this study, *E. hirta* ethanol extract depicted the best antioxidant activity in all antioxidant assays. It is because the *E. hirta* ethanol extract has notably more TPC and TFC than other extracts (Figure 2). Both the extraction technique and the use of different solvents affect the extraction results and antioxidant effect. The efficacy of a 70% ethanol-water solution as a solvent lies in its ability to extract a wide array of antioxidants (polarity index of ethanol ~5.2 and water ~9.0). Its polarity profile allows it to efficiently dissolve medium and highly polar compounds, including various phenolic acids, flavonoids, and tannins [26]. The mechanism involves the formation of hydrogen bonds between the solvent's molecules and the antioxidant compounds' –OH groups, which pulls them into the solution [27]. Consequently, this method yielded an extract with the greatest concentration and variety of active antioxidants. This high quality was confirmed by its potent DPPH radical scavenging ability, requiring a very low IC_{50}.

The utility of ethyl acetate in extraction is defined by its mid-range polarity (polarity index ~0.1). While it proficiently pulls mid-polarity flavonoids and phenolics of lower polarity from a mixture, it will overlook highly polar constituents. These, such as tannins and simple phenolic acids, are instead soluble in polar solvents like 70% aqueous ethanol [28]. While the extraction process was able to enrich specific compounds, the overall quantity of active antioxidants remained far lower than in an ethanolic extract. Consequently, this extract demonstrated activity only at a substantially higher concentration, matching the efficacy of its 70% ethanol-based counterpart.

Due to its extremely low polarity index of approximately 0.1, n-hexane is a profoundly nonpolar, aprotic solvent. This characteristic renders it ineffective at solubilizing polar antioxidant molecules, including those from the phenolic and flavonoid families, as their hydrophilic hydroxyl groups exhibit no chemical affinity for the hydrophobic hexane medium. Consequently, its extraction profile is dominated by nonpolar constituents like lipids, fixed oils, waxes, chlorophyll, and certain terpenoids [29]. Although some terpenoids possess antioxidant activity, they are generally far less effective in radical scavenging assays, such as the DPPH assay, than polyphenols. They are often not retrieved in a bioactive state by hexane [30]. The resulting extract, therefore, contained minimal quantities of chemical species that could donate a hydrogen atom to stabilize the DPPH radical. It was directly evidenced by the exceptionally high IC50 value, confirming the extract's practical inactivity at standard concentrations.

The findings of this study underscore a fundamental principle in phytochemistry: the extraction solvent exerts a profound influence on the yield of bioactive compounds and, consequently, on the measured antioxidant activity of plant extracts. Our results demonstrate that the ethanolic extract of *E. hirta* exhibited superior antioxidant potency, as measured by DPPH and $ABTS^+$ scavenging assays and reducing power, compared to extracts obtained using n-hexane and ethyl acetate. This efficacy is directly correlated with its significantly higher total phenolic content (TPC) and flavonoid content (TFC). This observation aligns with and substantiates the work of Nouioura et al. [31] on *Ferula communis* L. fruit and Fadhlillah et al. [32] on *Citrus reticulata* Blanco peel, who similarly found ethanolic extracts to be the most effective. However, a critical comparison moves beyond mere agreement. The consistency of this finding across such taxonomically diverse species—a Mediterranean Apiaceae (Ferula), a Rutaceae citrus fruit (Citrus), and our subject, a widespread Euphorbiaceae herb (Euphorbia)—suggests a universal trend rather than a species-specific phenomenon. It powerfully reinforces the axiom that ethanol, due to its balanced polarity, is a broadly effective solvent for a broad spectrum of antioxidant phytochemicals.

While our results confirm the general superiority of ethanol, a critical analysis must acknowledge that the "best" solvent is ultimately defined by the target bioactive. Previous studies on *E. hirta* itself have reported compelling findings using other solvents. For instance, research has identified potent anti-inflammatory and antimicrobial compounds in *E. hirta*'s dichloromethane and chloroform extracts, which are effective against more nonpolar constituents, such as terpenes and phorbol esters (e.g., see work on euphol and other triterpenes). Therefore, our conclusion that ethanol is "best" is context-specific to antioxidant activity linked to phenolics. For other therapeutic endpoints, a different solvent might be optimal. It highlights the importance

of a bioassay-guided fractionation approach rather than a one-size-fits-all method.

Furthermore, our study utilizes a maceration technique. Other extraction methods, such as Soxhlet extraction, ultrasound-assisted extraction (UAE), or supercritical fluid extraction (SFE), could significantly alter the efficiency of both ethanol and the other solvents. For example, the UAE can enhance the yield of antioxidants from E. hirta using ethanol in a shorter time and at a lower temperature, thereby preserving heat-labile compounds. A critical comparison must therefore consider that the solvent's performance is also modulated by the extraction technique employed.

Correlation Between TPC and TFC with Antioxidant Activity.

The presence of secondary metabolites determines a plant extract's antioxidant effect. Phenolics and flavonoids are types of phytochemicals known to have powerful antioxidant effects. Pearson's correlation was employed to evaluate the correlation between TPC, TFC, and antioxidant effects. The findings can be observed in Table 5.

A very strong positive correlation was observed between both TPC and TFC and the FRAP assay results ($p<0.01$), with remarkably high correlation coefficients (r-values) of 0.999 and 0.991, respectively. In practical terms, this near-perfect correlation means that simply measuring the TPC or TFC of these plant extracts provides an exceptionally accurate prediction of their ferric-reducing antioxidant power (as measured by FRAP). It is highly significant for applications where a rapid, cost-effective method for estimating this specific type of antioxidant activity is needed, as quantifying TPC/TFC is often simpler than running full antioxidant assays.

Conversely, TPC and TFC exhibited significant negative correlations with the DPPH and $ABTS^+$ radical scavenging assays ($p < 0.05$). The correlation coefficients for TPC and TFC with DPPH scavenging activity were -0.721 and -0.776, respectively, and with ABTS+ scavenging activity were -0.628 and -0.690, respectively. It is crucial to interpret this negative correlation correctly. In these assays, antioxidant activity is expressed as IC_{50}, which is the concentration required to scavenge 50% of the radicals. A lower IC_{50} value indicates a more potent antioxidant effect. Therefore, the negative correlation signifies that higher values of TPC and TFC are strongly associated with lower IC_{50} values. It means that extracts richer in phenolics and flavonoids are significantly more potent and efficient at quenching free radicals, requiring a smaller dose to achieve the same effect.

The practical implication of these strong correlations is substantial. It confirms that TPC and TFC can serve as reliable, preliminary biomarkers for the overall antioxidant potential of plant extracts. For industries involved in developing natural antioxidant products—such as functional foods, nutraceuticals, cosmetics, and preservatives—this means that high-throughput screening of plant materials can first focus on measuring TPC and TFC. It allows researchers to quickly identify the most promising candidate extracts for further, more resource-intensive testing and development, thereby streamlining the research and development process and reducing costs.

Table 5. Correlation between TPC, TFC, and antioxidant activity

	TPC	TFC	FRAP	DPPH	ABTS
TPC	1	0.996**	0.999**	-0.721*	-0.628*
TFC		1	0.991**	-0.776*	-0.690*
FRAP			1	-0.688*	-0.590
DPPH				1	0.992*
ABTS					1

Significance levels: $p < 0.05$ (*) and $p < 0.01$ (**), two-tailed.

The findings of our study align with and are reinforced by existing literature. Other studies have similarly demonstrated a strong connection between antioxidant activity, TPC, and TFC. For instance, Sukairi et al. [33] reported a very strong correlation between TPC, TFC, and the %inhibition of DPPH scavenging for *Piper sarmentosum* extract. The association of TPC and TFC in 12 native Indonesian plants with antioxidant properties was investigated by Muflihah et al. [34]. They demonstrated that TPC, DPPH, and H_2O_2 radical scavenging activity (%inhibition) had a substantial positive correlation. Additionally, they showed a significant positive relationship between TPC and the percentage of inhibition of H_2O_2 radical scavenging, as well as a moderately positive relationship between TPC and the rate of inhibition of DPPH radical scavenging. The consistency of these findings across different studies and plant species underscores the fundamental role these phytochemicals play in conferring antioxidant properties and validates the use of correlation analysis to guide practical research and application.

CONCLUSION

In summary, this study demonstrates that the extraction solvent has a profound impact on the recovery of bioactive compounds from *E. hirta*, with the ethanolic extract yielding the highest total phenolic and flavonoid content and, consequently, the most potent antioxidant activity *in vitro* compared to the ethyl acetate and n-hexane extracts. It confirms ethanol's efficacy as a polar solvent for solubilizing the plant's antioxidant principles, validating its use in traditional preparations and aligning with findings from other medicinal plants. However, these results cannot directly predict *in vivo* antioxidant efficacy or therapeutic potential due to the complexities of

absorption, metabolism, and bioavailability in a living system. Furthermore, the absence of comprehensive phytochemical profiling means the specific phenolic acids, flavonoids, or other compounds responsible for the observed activity remain unidentified. Therefore, future research should be conducted to understand the pharmacological value of *E. hirta*, including phytochemical characterization, *in vivo* validation, and exploration of the antioxidant mechanism.

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