



Effects of Solvent Type and Simplicia-to-Solvent Ratio on the Phenolics, Flavonoids, and Antioxidant Activity of Torch Ginger (*Etilingera elatior*) Fruit

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ABSTRACT

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Torch ginger (*Etilingera elatior*) is an aromatic plant commonly used as a spice in food. It contains key phytochemicals such as phenolics, and flavonoids with potent antioxidant activity. This study aimed to investigate the effect of extraction condition (solvent type and Simplicia-to-Solvent ratio) on the total phenolic content, total flavonoid content, and antioxidant properties of torch ginger fruit (TFG). Nine different extracts combinations of TGF were obtained, with three solvents (70% ethanol, ethyl acetate, and n-hexane), and three powdered Simplicia-to-Solvent ratios (1:10, 1:20, and 1:30). The total phenolic, and total flavonoid contents of the different extracts were determined using standard procedures. The antioxidant activity was evaluated by the 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay. The results showed that ethanol extract at a Simplicia-to-Solvent ratio of 1:30 contained the highest total phenolic, and total flavonoid contents of 108.43 ± 2.33 mg GAE/g extract and 1.22 ± 0.08 mg QE/g extract, respectively. In addition, the ethanol extract at a Simplicia-to-Solvent ratio of 1:30 exhibited the most potent antioxidant activity with IC_{50} of 76.157 ± 1.461 μ g/mL. In conclusion, ethanol solvent at a Simplicia-to-Solvent ratio of 1:30 is the optimal extraction condition needed to achieve the highest total phenolic and flavonoid contents from TGF, while also providing the highest antioxidant activity.

Keywords: *Etilingera elatior*, Extraction, Flavonoids, Phenolics, Antioxidants.

Introduction

Free radicals are reactive molecules with one or more unpaired electrons. Free radicals are formed during cellular metabolic reaction in mitochondria or from the fragmentation of molecules resulting in unpaired electron-bearing fragments.¹ Examples of free radicals are nitric oxide (NO•), superoxide (O^{2•-}), nitrogen dioxide (NO₂•), hydroxyl (OH•), peroxy (ROO•) and lipid peroxy (LOO•) radicals. These reactive species interact with key molecules in human body like proteins, lipids, and DNA, leading to various diseases, and contributing to the aging process. Endogenous free radicals are produced through biological processes like excessive exercise, cellular aging, inflammation, and immune responses, while exogenous radicals are generated by pollution, smoke, transition metals, radiation, and certain drugs, such as gentamicin and cyclosporine.²⁻⁶ In order to prevent free radical-related health problems, researchers are actively exploring the potential of antioxidants, especially from natural sources in mitigating the effect of free radicals. Antioxidants have the potential to neutralize free radicals, which is why they are commonly used to preserve food, and help prevent diseases like cancer, and cardiovascular conditions. The presence and quantity of key phytochemical compounds with antioxidant activity in an extract are key determinant of their antioxidant potency. Several factors such as geographical location, drying process, and the extraction procedure can influence the amount of phytochemicals extracted from plants.

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When it comes to the extraction method, important factors to consider include the equipment used, the type of solvent, as well as the extraction time and temperature. Ultrasound-assisted extraction is one of the best methods of extraction of phytochemicals from plants. This method uses ultrasound to disrupt plant cells, allowing the compounds to be more easily extracted by the solvent. Additionally, the bubbles formed by the ultrasound prevent solvent saturation, which further enhances the extraction process. Several studies have been conducted to improve the yield of extracted compounds. For example, it was observed that heating the sample combined with ultrasound extraction resulted in higher levels of phenolics, flavonoids, and ABTS radical scavenging activity in several samples.⁷ Another research found that ultrasound-assisted extraction is an effective extraction method for obtaining high levels of phenolics, caffeine, and chlorogenic acid from coffee silverskin.⁸ Additionally, the type of solvent used for extraction has been reported to significantly influence the type and concentration of important phytochemicals obtained. Several studies found that the mixture of organic solvent and water (for example 50% ethanol or 50% acetone) yield a higher concentration of phenolics, saponins, and flavonoids. Furthermore, these solvent mixtures also produce extracts with strong antioxidant activity.^{9,10} Torch ginger (*Etilingera elatior*) is a plant native to the Sulawesi region, commonly used as a cooking spice and in beverages. Research has been conducted on various parts of the torch ginger plant. The leaves, in particular, have been found to contain bioactive compounds such as chlorogenic acid, phenolic compounds, and amino acids, which contribute to their antioxidant activity. Additionally, the leaves exhibit inhibitory effects against the tyrosinase enzyme, making them a potential candidate for anti-aging preparations.¹¹ In addition, the flower buds and flowers of torch ginger also contain bioactive compounds such as α -pinene, farnesyl alcohol, dodecanol, caryophyllene, hexadecanol, ascorbic acid, and pentadecanol. These compounds exhibit strong activity against DPPH free radicals and demonstrate antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes*.¹²⁻¹⁴ Furthermore, torch ginger fruit has been found to contain alkaloids, phenolics, flavonoids, tannins, and terpenoids such as

1-methyl-4-methylenecyclohexane, 6-azabicyclo [3.2.1] octane, and hexadecanoic acid. These compounds exhibit inhibitory activity against the growth of *E. coli* and *S. aureus* *in vitro*.¹⁵

The present study was conducted to investigate the effects of solvent type and simplicia-to-solvent ratio on the yield, phenolic, and flavonoid contents, as well as the antioxidant activity of torch ginger fruit (TGF).

Materials and Methods

Preparation of sample

Fresh torch ginger fruit (TGF) was purchased from the local market on 28th February 2024 at Palopo Regency, South Sulawesi Province, Indonesia (Latitude: 3.0016°S, Longitude: 120.1970°E) (Figure 1A). Sample species was confirmed at laboratory of pharmacognosy, at Hasanuddin University (Document No. 038/SKIT/Farmakognosi/XI/2024). Specimen voucher is also stored at the same laboratory with reference No. 038-SKIT-b. Fresh TGF was washed, drained, and dry sorted manually. It was then dried in the oven at 60°C for 24-48 hours and ground using a blender. The resulting coarse powder (Figure 1B), was weighed in about 10 g portions and put into a amber coloured bottle for the extraction process.



Figure 1: A) Fresh torch ginger fruit, B) Torch ginger fruit dry powder

Extraction

The extraction conditions were designed using nine different combinations, with three solvents (70% ethanol, ethyl acetate, and n-hexane), and three simplicia-to-solvent ratios (1:10, 1:20, and 1:30). TGF powder (10.4 g each) were placed into the different amber coloured bottles, and were sonicated for 60 min. The extracts were filtered, and the filtrates were concentrated using rotary evaporator at 60°C and air dried above the water bath. The dried extracts were transferred into amber coloured vials, and stored at 4-8°C until used for bioassay.^{16,17}

Quantification of total polyphenolic compounds

The total polyphenolic concentration in each extract was measured spectrophotometrically using the Folin-Ciocalteu method, and gallic acid as the standard. Approximately 10 mg of each extract was weighed and dissolved in suitable solvents to prepare 1000 µg/mL stock solution. Each stock solution was mixed with 7.5% of Folin-Ciocalteu reagent and 1% of NaOH. The reaction mixture was incubated in the dark for 60 min, and then the absorbance was measured using a UV-Vis spectrophotometer (Shimadzu, Japan) at 762 nm. The quantification was performed in triplicate. The total polyphenolic compounds were expressed as milligram gallic acid equivalent per gram of the extract (mg GAE/g extract).^{16,18}

Quantification of total flavonoid compounds

The total flavonoid concentrations in each extract was measured spectrophotometrically, with quercetin as the standard. Approximately 50 mg of each extract was weighed and dissolved in suitable solvents to prepare 5000 µg/mL stock solution. The stock solution was mixed with 10% AlCl₃ and 1 M sodium acetate. The reaction mixture was then incubated for 30 min, and the absorbance was measured using a UV-Vis spectrophotometer (Shimadzu, Japan) at 423 nm. The quantification was done in triplicate, and the total flavonoid content was expressed as

milligram quercetin equivalent per gram of the extract (mg QE/g extract) (19).

DPPH free radical scavenging activity

The ability of TGF extracts to neutralize 2,2-diphenyl-1-picryl hydrazyl (DPPH) radicals was determined spectrophotometrically using a microplate reader (Biotek ELx808, China). Five different concentrations of the sample were mixed with 70 µL of DPPH solution in methanol (0.1 mM), and then methanol was added up to a total volume of 200 µL in a 96-well plate. The plate was incubated in the dark for 30 min. After incubation, the absorbance was measured at 515 nm. The DPPH scavenging potency was calculated using the following equation:

$$\% \text{ Scavenging} = \frac{\text{Abs control} - \text{Abs extract}}{\text{Abs control}} \times 100$$

Where;

“Abs control” is the absorbance of DPPH and methanol (without extract), “Abs extract” is the absorbance of the test using TGF extracts after 30 min of incubation with DPPH.

IC₅₀ was calculated as the concentration of extracts which resulted in the 50% inhibition of DPPH radical.¹⁹

Statistical analysis

All measurements were conducted in triplicates and the results were expressed as mean ± standard deviation (SD). Analysis of variance was performed using GraphPad Prism version 9 software with a one-factor design to assess the phenolic content, flavonoid content, and DPPH radical scavenging activity.

Results and Discussion

In the present study, the influence of three solvents with different polarities and three different powdered plant simplicia-to-solvent ratios on extract yield, total phenolic content, total flavonoid content, and antioxidant activity of TGF extracts was investigated.

Effect of solvent, and simplicia-to-solvent ratio on extract yield

The extraction process started with the preparation of powdered plant material, where torch ginger fruit (TGF) samples were thoroughly cleaned to remove dust and dirt from the surface. The cleaned TGF was dried at 60°C for 48 hours to reduce its water content, thereby minimizing the risk of bacterial and fungal growth. Additionally, the drying step facilitates easier pulverization.^{20,21} The dried TGF was then ground using a blender to increase the surface area, ensuring better contact between the powdered TGF sample, and the solvent during the extraction process.

Extraction was performed using three solvents of varying polarity: 70% ethanol, ethyl acetate, and hexane. Additionally, the ratio of powdered sample to solvent was varied at 1:10, 1:20, and 1:30 to determine the best solvent and Simplicia-to-Solvent ratio that would yield the highest extract with the greatest phytochemical content. The extract yields were calculated and the results are presented in Table 1. Data from three extraction replicates for each treatment showed that different solvents produced varying extract yields. Extraction with 70% ethanol at a Simplicia-to-Solvent ratio of 1:20 resulted in the highest yield of 14.59%, indicating that ethanol was the most effective solvent for dissolving the phytochemical compounds from TGF (*E. elatior*). In contrast, n-hexane extract yielded the lowest amount, with just 0.081% at a 1:30 Simplicia-to-Solvent ratio. Statistical analysis (Figure 2a) revealed that the solvent type had a highly significant effect on the percentage yield ($p < 0.0001$), except for the ethyl acetate and n-hexane at a 1:20 Simplicia-to-Solvent ratio, where no significant difference was observed. This outcome may be due to the ability ethanol to dissolve the polar secondary metabolites of TGF more effectively. Polar solvents are generally more efficient at recovering compounds in the extraction process compared to non-polar solvents. Previous studies have shown that extraction yields decreased as solvent polarity decreased.²²⁻²⁴ However, as presented in Figure 2b, the different Simplicia-to-Solvent ratios did not have a significant impact on the percentage yield, except for the n-hexane extract, where the 1:10 Simplicia-to-Solvent ratio

produced a significantly different percentage yield compared to the 1:20 ratio.

Total phenolic contents

The total phenolic content varied among the different extracts, as shown in Table 2. The determination of the polyphenol content of each extraction treatment was aimed at identifying and comparing the type of solvent and the Simplicia-to-Solvent ratio that would produce the highest polyphenol content. This is significant because polyphenols play a crucial role in the antioxidant and antibacterial activities of plants. The abundance of hydroxyl and phenolic groups in polyphenol compounds helps neutralize free radicals, which can be harmful to human health.¹⁷

Table 1: Percentage yields of TGF extracts under different extraction conditions

Sample	Sample to solvent ratio	Yield (%)
EE	1:10	12.204 ± 1.031
	1:20	14.599 ± 0.806
	1:30	14.332 ± 1.059
EAE	1:10	3.785 ± 0.370
	1:20	3.251 ± 0.759
	1:30	4.090 ± 0.226
HE	1:10	0.752 ± 0.203
	1:20	1.352 ± 0.573
	1:30	0.830 ± 0.081

Values are mean ± SD of triplicate measurements. TGF = Torch ginger fruit, EE = Ethanol extract, EAE = Ethyl acetate extract, HE = n-Hexane extract

Table 2: Total polyphenol contents of TGF extracts under different extraction conditions

Sample	Sample to solvent ratio	Total polyphenol content (mg GAE/g extract)
EE	1:10	99.28 ± 3.463
	1:20	104.18 ± 4.672
	1:30	108.43 ± 2.327
EAE	1:10	16.38 ± 1.946
	1:20	22.69 ± 0.889
	1:30	25.51 ± 0.539
HE	1:10	2.06 ± 0.069
	1:20	2.13 ± 0.161
	1:30	2.24 ± 0.113

Values are mean ± SD of triplicate measurements. TGF = Torch ginger fruit, EE = Ethanol extract, EAE = Ethyl acetate extract, HE = n-Hexane extract

Based on the results obtained (Table 2), the ethanol extract with a ratio of 1:30 showed the highest polyphenol content of 108.43 ± 2.33 mg GAE/g extract, which was 50 times greater than that of the hexane extract at all Simplicia-to-Solvent ratio. Figure 3A illustrates the polyphenol content extracted using three different solvents and three simplicia-to-solvent ratios. Generally, the analysis of the results show that ethanol had an extremely significant ($p < 0.0001$) effect on polyphenol content, indicating that ethanol is more effective at extracting polyphenolic compounds compared to ethyl acetate and n-hexane. This suggests that ethanol should be considered as the preferred solvent when the goal is to maximize polyphenol extraction from a sample. Furthermore, as presented in Figure 3B, analysis of the effect of simplicia-to-solvent ratio revealed that the higher the amount of solvent used compared to the powdered plant materia, the higher the polyphenol content extracted.

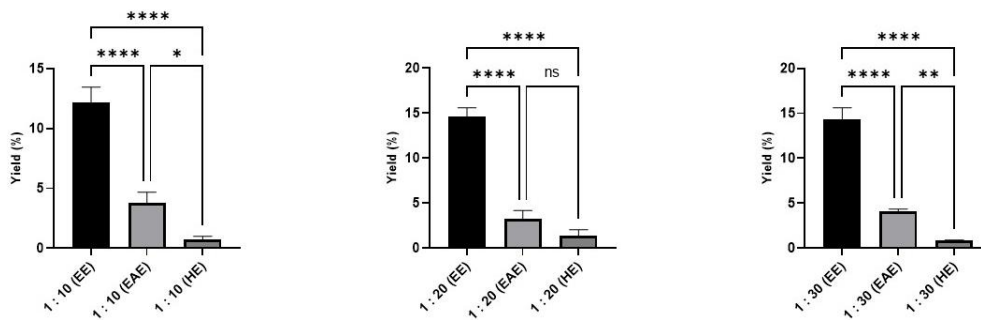


Figure 2A: Effect of solvent type on extract yield of torch ginger fruit. Note: ns: non-significant; ** significant at $p < 0.01$; ***significant at $p < 0.001$.

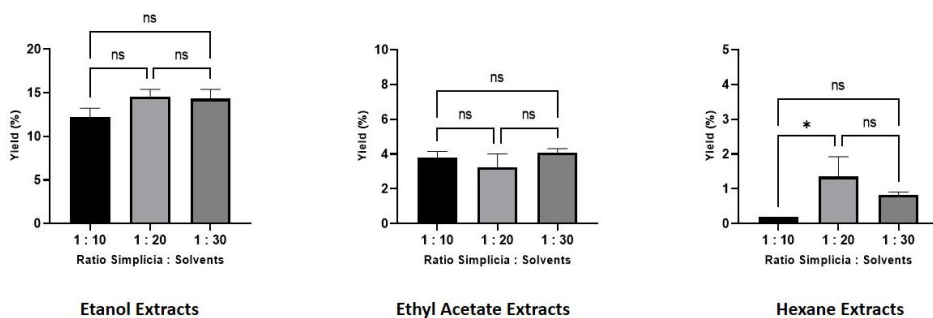


Figure 2B: Statistical analysis of the effect of Simplicia-to-Solvent ratio on extract yield of torch ginger fruit. Note: ns: non-significant; ** significant at $p < 0.01$; ***significant at $p < 0.001$.

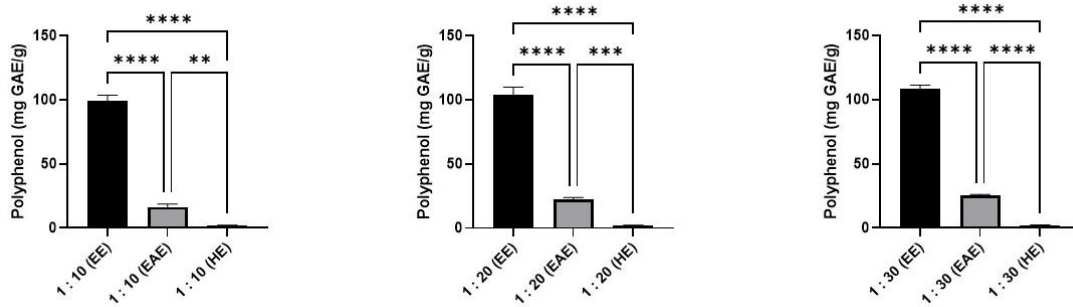


Figure 3A: Statistical analysis of the effect of solvent type on total polyphenolic content of torch ginger fruit extract. Note: ***significant at $p < 0.001$.

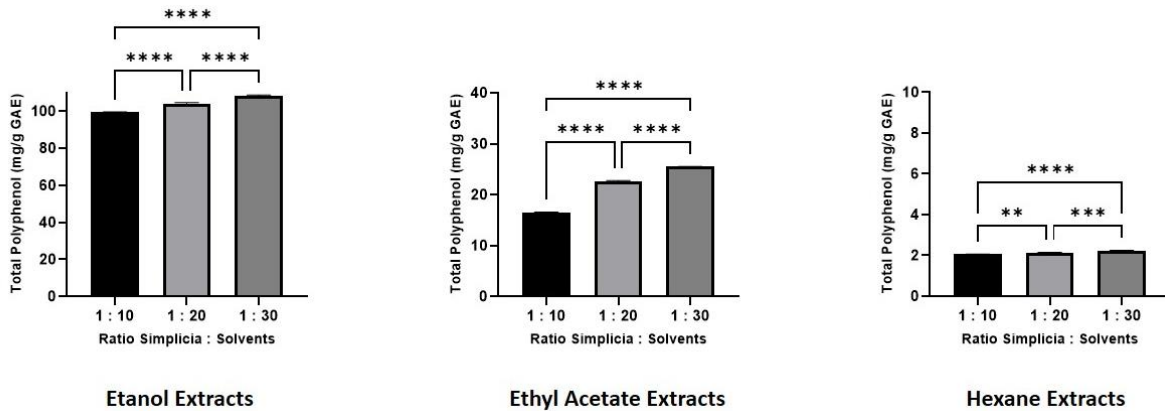


Figure 3B: Statistical analysis of the effect of Simplicia-to-Solvent ratio on total polyphenolic content of torch ginger fruit extract. Note: ***significant at $p < 0.001$.

Total flavonoid contents

The total flavonoid content varied among the different extracts as presented in Table 3. Flavonoids play a crucial role as antioxidant, their structure, which is made up of numerous conjugated double bonds, hydroxyl groups, and phenolic groups, enables them to effectively scavenge free radicals. Additionally, flavonoids can transfer hydrogen, activate antioxidant enzymes, and inhibit oxidation processes.²⁵

Table 3: Total flavonoid contents of TGF extracts under different extraction conditions

Sample	Sample to solvent ratio	Total flavonoid content (mg QE / g extract)
EE	1:10	1.10 ± 0.042
	1:20	1.13 ± 0.037
	1:30	1.22 ± 0.079
EAE	1:10	0.61 ± 0.020
	1:20	0.58 ± 0.021
	1:30	0.68 ± 0.018
HE	1:10	0.19 ± 0.001
	1:20	0.21 ± 0.000
	1:30	0.22 ± 0.005

Values are mean \pm SD of triplicate measurements. TGF = Torch ginger fruit, EE = Ethanol extract, EAE = Ethyl acetate extract, HE = n-Hexane extract

Table 3 shows that the ethanol extract with a 1:30 Simplicia-to-Solvent ratio had the highest flavonoid content, with a value of 1.22 ± 0.08 mg QE/g extract, which was approximately 10 times higher than that of the n-hexane extract across all treatments. Figures 4a and 4b present the results of flavonoid contents of TGF extracts, prepared using three different solvents and three different Simplicia-to-Solvent ratios. Figure 4A shows that ethanol had an extremely significant ($p < 0.0001$) effect on the flavonoid content of TGF extract compared to n-hexane and ethyl acetate. Flavonoids, a group of secondary metabolites with potent antioxidant activity, commonly occur as glycosides with sugar molecules attached to their structure. This characteristic makes them to be more effectively extracted in aqueous ethanol solvents.²⁶ Therefore, ethanol can be considered as the best solvent of choice for dissolving flavonoid compounds in a sample. Additionally, the TGF may consist of polar flavonoids that are easily extracted in the mixture of ethanol and water. Further analysis of the effect of the Simplicia-to-Solvent ratio on the flavonoid content of TGF extract revealed that the higher amount of solvent compared to powdered plant sample results in a greater concentration of flavonoid extracted (Figure 4B). In this study, the Simplicia-to-Solvent ratio of 1:30 resulted in the highest flavonoid content, making this method the most effective for maximizing flavonoid extraction.

DPPH radical scavenging activity

Antioxidants play a crucial role in maintaining human health by preventing cellular damage caused by free radicals. They employ several mechanisms, such as radical scavenging, electron donation, and hydrogen transfer. The DPPH radical scavenging assay is one of the established methods for evaluating antioxidant activity. DPPH is a dark purple powder that can be dissolved in ethanol or methanol to form a dark purple solution. Antioxidant compounds can reduce stable DPPH radicals by donating hydrogen to them, resulting in a yellow solution due to the formation of DPPH-H (the reduced form of DPPH).^{27,28}

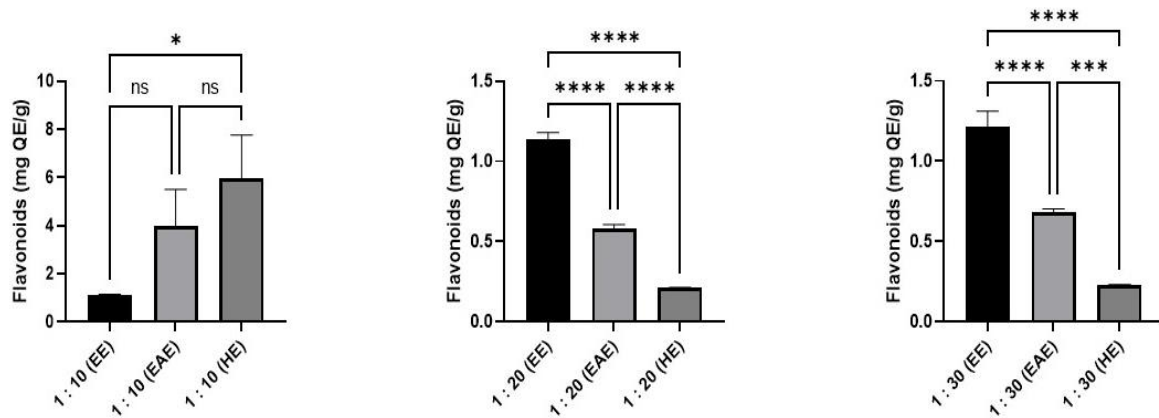


Figure 4A: Statistical analysis of the effect of solvent type on total flavonoid content of torch ginger fruit extract. Note: ns: non-significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; ***.significant at $p < 0.001$

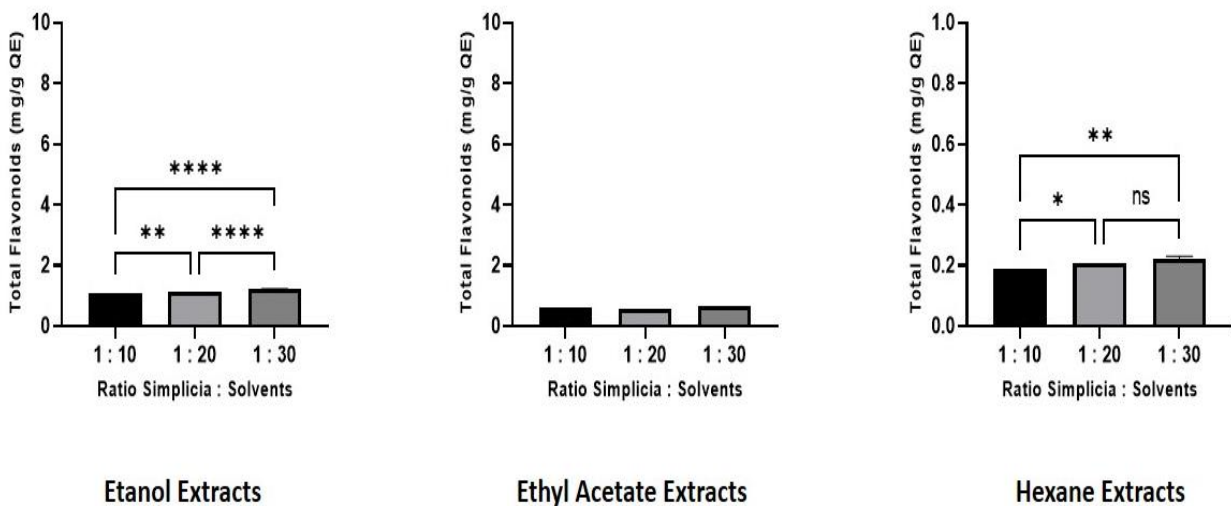


Figure 4B: Statistical analysis of the effect of Simplicia-to-Solvent ratio on total flavonoid content of torch ginger fruit extract. Note: ns: non-significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; ***.significant at $p < 0.001$

This process leads to a decrease in the absorbance of the DPPH-extract solution.

Table 4 shows the antioxidant activity of ethanol extract, ethyl acetate extract, and n-hexane extract of TGF with different Simplicia-to-Solvent ratios. The ethanol extract with a simplicia-to-solvent ratio of 1:30 exhibited the lowest IC_{50} value of $76.157 \pm 1.461 \mu\text{g/mL}$ for DPPH radical scavenging activity, while the n-hexane extract with a simplicia-to-solvent ratio of 1:20 gave the highest IC_{50} value of $2662.037 \pm 10.632 \mu\text{g/mL}$ for DPPH radical scavenging activity. This indicates that ethanol extract exhibited the strongest DPPH radical scavenging activity among the tested samples, whereas n-hexane extract demonstrated the lowest ability to neutralize DPPH radicals. However, when compared to ascorbic acid, which had an IC_{50} of $6.18 \mu\text{g/mL}$, the antioxidant activity of ethanol extract is approximately 12 times lower than that of ascorbic acid. This finding is supported by the significance analysis presented in Figure 5A, which shows that the ethanol extract had a highly significant inhibition ($p < 0.0001$) against DPPH radicals compared to ethyl acetate, and n-hexane extracts. Additionally, Figure 5B illustrated that the Simplicia-to-Solvent ratio of 1:30 produced a better antioxidant effect than the other ratios. There is a strong correlation between polyphenolic content and antioxidant activity; extracts with higher levels of polyphenolic compounds exhibit stronger antioxidant effects.

Table 4: DPPH Free radical scavenging activity of TGF extracts

Sample	IC ₅₀ of Antioxidant Activity ($\mu\text{g/mL}$)		
	Sample to solvent ratio		
	1:10	1:20	1:30
EE	77.096 ± 1.023	87.01 ± 2.022	76.157 ± 1.461
EAE	334.68 ± 15.450	267.947 ± 8.238	315.46 ± 6.735
HE	2508.633 ± 8.339	2662.037 ± 10.632	2511.733 ± 3.052

Notes: Under the same condition, ascorbic acid had an IC_{50} of $6.18 \mu\text{g/mL}$

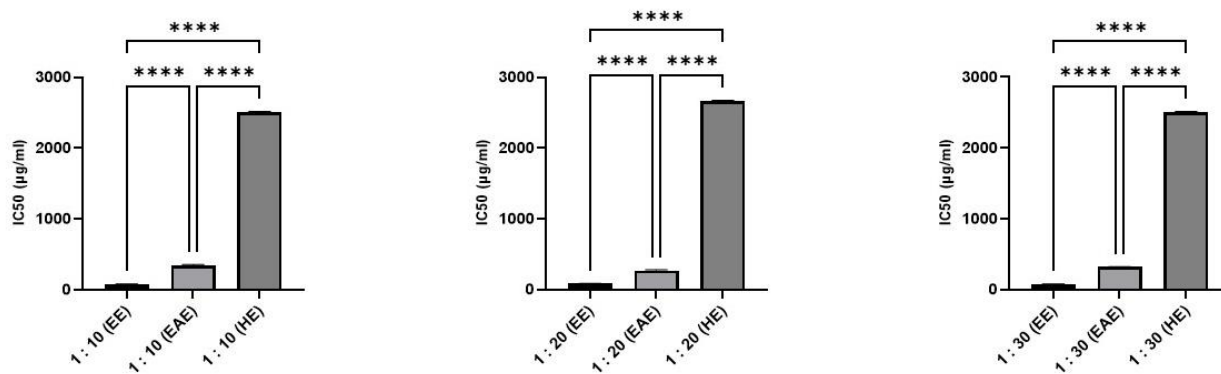


Figure 5A: Statistical analysis of the effect of solvent type on antioxidant activity of torch ginger fruit extract

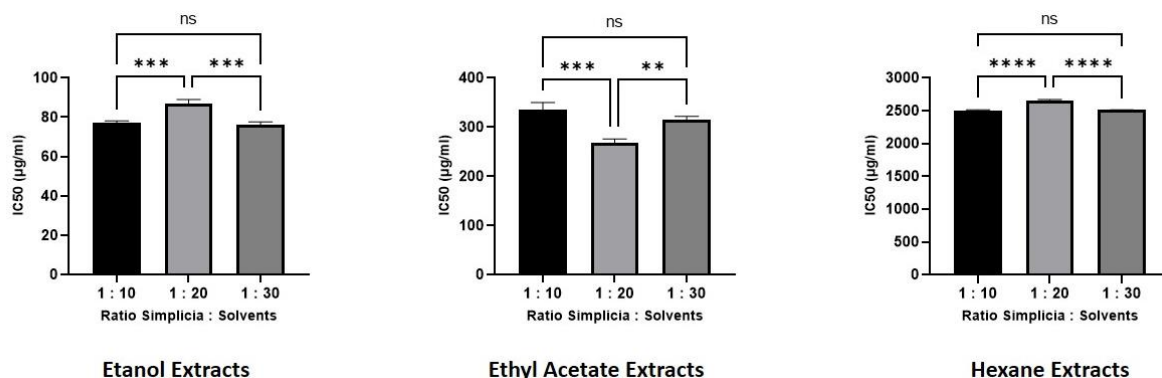


Figure 5B: Statistical analysis of the effect of Simplicia-to-Solvent ratio on antioxidant activity of torch ginger fruit extract

The hydroxyl and phenolic groups in polyphenol compounds can effectively neutralize free radicals that potentially affect human health.¹⁷ Furthermore, there is a significant correlation between flavonoids and antioxidant activity. The ethanol extract which contains the highest amount of flavonoid requires the lowest concentration to scavenge DPPH radical.^{29,30}

Conclusion

This study found that polar solvent, such as aqueous ethanol, play a significant role in extracting higher concentrations of polyphenolics and flavonoids from TGF, resulting in increased antioxidant activity. This effectiveness may be due to the high number of hydroxyl groups present in the structure of phenolics and flavonoids. Furthermore, a simplicia-to-solvent ratio of 1:30 is generally the best for extracting these compounds.

Conflict of Interest

The authors declared no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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