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Greener approaches to improve phenolic acid composition and antioxidant activity of different cultivation methods of *Zingiber officinale* Roscoe extracts

[1, *]Nur Diyana, A., [2]Nor Azlan, S.N.,[1]Mohd Lazim, M.I., [1]Hussin, A., [1]Mansor, A. and [1]Ibrahim, A.H.

[1]*Enzyme and Fermentation Technology Programme, Food Science and Technology Research Centre, MARDI, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia*
[2]*Biomolecular Science, Faculty of Applied Sciences, Universiti Teknologi MARA, Jalan Ilmu 1/1, 40450 Shah Alam, Selangor, Malaysia*

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Abstract

Zingiber officinale Roscoe, known as ginger (Bentong variety), is an essential spice ingredient used in food and condiments due to its refreshing aroma and intense flavour. The bioactive constituents found in ginger influence the numerous pharmacological effects, including antioxidant, analgesic, anticancer, antipyretic and anti-inflammatory. This work proposed a greener approach than the conventional plant matrices extraction to promote safe, non-toxic, and environment-friendly products. In this study, three different extraction methods: a) water (WE), b) hydrothermal-assisted (HAE) and c) enzymatic-assisted (EAE), have been applied to measure the potential of both ginger extracts from different cultivation methods using fertigation technology and conventionally slope-grown. To ascertain the biological content of the extracts, the phenolic acid composition was quantified using ultra-performance liquid chromatography (UPLC). The total phenolic content (TPC) was determined using the Folin-Ciocalteu method, while the antioxidant activities were analysed using ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The results obtained showed that ferulic acid was significantly highest in EAE extracts of both ginger (fertigation and slope-grown cultivation) at 1.165±0.118 and 0.610±0.062 mg/g. Moreover, EAE of both ginger extracts also found to be significantly highest in TPC (267.481±0.404 mg GAE/g and 247.837±1.675 mg GAE/g), and FRAP value (741.867±2.139 mg AAE/g and 461.733±0.231 mg AAE/g), respectively. However, the highest free radical scavenging activity of fertigated ginger extract through HAE was 134.735±0.021 mg AAE/g. Meanwhile, EAE of the sloped-grown ginger extract was highest at 141.293±0.707 mg AAE/g. This study demonstrates that EAE can be an effective approach in enhancing the TPC and antioxidant activity of ginger extracts. Ginger may therefore be suggested as a more greener and natural antioxidant to lessen oxidation in food products.

1. Introduction

Zingiber officinale Roscoe, indigenously known as 'Halia Bentong' in Malaysia, is a member of the Zingiberaceae family with several interesting bioactive constituents and possesses therapeutic characteristics (Ghasemzadeh *et al*., 2010a). According to the official portal of the Department of Statistics Malaysia, ginger reached 81.5% Import Dependence Ratio (IDR) among other crops in 2020. This indicates a high consumption of ginger among Malaysians. In furtherance of the above statement, ginger has been extensively utilised and become a prominent culinary flavouring and condiment in local cuisine. The main constituent of ginger are carbohydrates (50-70%), lipids (3-8%), terpenes (zingiberene, β-bisabolene, α-farnesene, β-sesquiphellandrene and α-curcumin) and phenolic compounds including gingerols, paradols and shogaol (Grzanna *et al.*, 2005; Prasad and Tyagi, 2015). In addition, additional amino acids, raw fibre, ash, protein, phytosterol, vitamins and minerals are present in ginger (Prasad and Tyagi, 2015). Findings by Tyler (1993), suggested that the aromatic constituent of ginger comes from zingiberene and bisabolene. Meanwhile, gingerols and shogaols are the major compounds that contribute to

*Corresponding author.
Email: *nurdiyana@mardi.gov.my*

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the pungent flavour of ginger (Prasad and Tyagi, 2015).

Ginger contains natural substances that help prevent or reduce cell damage caused by free radicals. Free radicals could induce oxidative stress and it can be prolonged to cause DNA damage and cell death. Antioxidant constituents can protect the human body from free radicals and reactive oxygen species (ROS) (Bae *et al*., 2016). The biological activity has frequently been described in connection with antioxidant capabilities, which might be connected to plant polyphenolic substances. Ginger has been reported to effectively protect against oxidative stress (Stoilova *et al*., 2007). In addition, accumulating studies have demonstrated that active ingredients like phenolic compounds are mainly associated with ginger's antioxidant activity (Mao *et al*., 2019). The amount of antioxidative phenolic compounds in natural sources are covalently bonded. Therefore, extraction methods were applied to the plant materials to enhance the antioxidant capacity (Xu *et al*., 2007).

Extraction is the process of separating active plant components or secondary metabolites from inert material by employing the appropriate method (Abubakar and Haque, 2020). Conventionally, to assure the extraction of a wide variety of compounds with various polarities, the serial exhaustive extraction technique comprises sequential extraction with solvents of increasing polarity from non-polar to more polar (water) (Nawaz *et al*., 2020). According to research, the polarity of the solvent has a substantial impact on the extract yield and antioxidant activity of phenolic chemicals in plant material (Nawaz *et al*., 2020). The conventional extraction method was recently replaced by alternative and greener techniques to minimise chemical usage and increase efficiency and selectivity to meet market demand and environmental laws and regulations (Chaves *et al*., 2020). According to Chemat *et al*. (2012), green extraction may be defined as the discovery and design of an extraction process as an alternative approach to minimise energy consumption, allow the use of alternative solvents and renewable natural resources, and ensure the safety and high quality of the extract. Due to its polarity, many extractants have been employed to extract the bioactive compound from plant materials, including water. For example, water has been applied to extract phenolics and antioxidants from ginger (Tohma *et al*., 2016). Besides extractant polarity, the extraction temperature and duration are critical determinants in antioxidants and phenolic extractability. Hydrothermal extraction has numerous advantages, including high recovery; it is faster and energy-efficient (Shinde and Shinde, 2017). On the other hand, enzymes are also utilised in bioactive compound extraction due to their effectiveness in biomolecule extraction and are considered a green extraction technique. The enzyme-assisted extraction approach uses specific enzymes to break down the cell wall of the plant materials to improve extraction yield (Marathe *et al*., 2017). Moreover, enzyme-assisted extraction benefits from extracting the maximum yields of phenols while also being safer and environmentally friendly (Shinde and Shinde, 2017). However, there is diversification of bioactive compounds found in plants, and their solubility qualities vary (Truong *et al*., 2019). Therefore, recommending an appropriate extraction approach for unique plant materials is often challenging.

To demonstrate the foundation of scientific knowledge and a better understanding of ginger, the properties of phenolic compounds and antioxidants must be evaluated. This study aimed to access the phenolic compound and antioxidant activities of different cultivation methods of ginger extract using greener approaches. This study offers three different types of extraction for both ginger cultivation methods (fertigation and slope-grown), which are water extraction (WE), hydrothermal-assisted extraction (HAE), and enzymatic-assisted extraction (EAE). The antioxidant activities and phenolic compound were investigated for each extract for both cultivation methods of ginger. The results may provide alternative and greener extracts of antioxidants and phenolic acid from ginger.

2. Materials and methods

2.1 Preparation of Gingiber officinale samples

Gingiber officinale, ginger of Bentong varieties from fertigation and slope-grown cultivation were freshly acquired from a different farm located in Sepang, Selangor and Bentong, Pahang. Both varieties of young ginger were harvested when they were roughly at six to seven months of maturation stage. Ginger samples were soaked and rinsed in running tap water for the cleaning process. It was then dried in a ventilated oven (FD-400, Tech-lab MFG Sdn Bhd, Malaysia) before being ground into 0.5 mm powder using an ultra-centrifugal mill (Retsch Zm200, Germany). Finally, the dried ginger samples were vacuum-sealed using a full well packaging system (DZ-400/2ES, Glarity Excel Sdn. Bhd., Malaysia) before being stored in a chiller at 5°C for further use.

2.2 Determination of Gingiber officinale extraction

Three different extraction processes were evaluated in the phenolic content and antioxidant activities: (i) water extraction (WE), (ii) hydrothermal-assisted extraction (HAE), (iii) enzyme-assisted extraction (EAE).

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2.2.1 Water extraction

Approximately 1 g of ginger was added in 50 mL of distilled water. Then, the mixture was incubated at 30°C, vigorously shaken at 160 rpm for 24 hrs using an incubator shaker (Innova 4000, New Brunswick Scientific, Germany). The extract was then centrifuged at 20°C, 10 000 rpm in 10 mins (Centrifuge Eppendorf AG 22331, Hamburg, Germany). It was then filtered using Whatman no. 1 filter paper and kept at -20°C for further use. All extractions were carried out in triplicate.

2.2.2 Hydrothermal-assisted extraction

Hydrothermal assisted extraction method involves high-temperature at atmospheric pressure. A total of 1 g of ground ginger powder was extracted using 50 mL of distilled water. The mixture was heated at 121°C, 0.1 MPa for 15 mins (ES-315-TOMY, Japan) before being incubated at 30°C, 160 rpm for 24 hrs using an incubator shaker (Innova 4000, New Brunswick Scientific, Germany). It was then centrifuged at 20°C, 10,000 rpm for 10 mins (Eppendorf AG, Germany) before filtered using Whatman no. 1 filter paper. The collected extract was kept at -20°C for further use. All extractions were carried out in triplicate.

2.2.3 Enzymatic-assisted extraction

The hydrolytic cellulase enzyme extraction was performed with slight modification, as described by Chen *et al.* (2011). The enzyme solution at the concentration of 1% was added into 1 g of ground ginger samples and immersed in 50 mL distilled water with adjusted pH at 7.00 using a certain amount of 0.5 M NaOH. Subsequently, the mixture was incubated at 50°C for 24 hrs at 160 rpm (Innova 4000, New Brunswick Scientific, Canada). The enzyme was deactivated by heating at 90°C for 10 mins before centrifuging at 20°C, 10,000 rpm for 10 mins (Eppendorf AG, Hamburg, Germany) and filtered using Whatman no. 1 filter paper. The extract was kept at -20°C for further usage. All extractions were carried out in triplicate.

2.3 Determination of total phenolic content

The total phenolic content was measured spectrophotometrically using Folin-Ciocalteu assay at absorbance 765 nm. Ginger extract was incorporated with a 5 ml Folin-Ciocalteu reagent (Merck, USA) and incubated at 30°C for 10 mins before being added with 4 ml of 7.5% sodium carbonate solution. After 2 hrs of incubation, the absorbance of the samples was measured. The calibration curve expressed the amount as gallic acid (Sigma-Aldrich) equivalents (mg GAE/g extract).

2.4 Determination of phenolic acid composition

The free phenolic acids in the samples were analysed using the Ultra Performance Liquid Chromatography (UPLC) Acquity (Waters) with a slight modification of Li *et al*. (2013). Approximately 1 µL aliquot of sample solution was separated using a reverse-phase analytical column (100 mm × 2.1 mm XBridge-BEH C18, 2.5 m, Waters) and photodiode array (PDA) detector at 30°C controlled temperature. The mobile phase was 0.1% formic acid (A) and methanol (B), with a 0.5 mL/min flow rate. Peak identification was accomplished by comparing retention times and UV spectra at 270, 305 and 325 nm with authentic compounds. Calibration curves generated by injecting known quantities of pure substances as external standards were used for quantification.

2.5 Determination of antioxidant activities

2.5.1 Ferric reducing antioxidant power

FRAP activity was measured spectrophotometrically. The assay was performed using a modification of Haida and Hakiman (2019). A FRAP dye working solution of 0.3 M acetic acid, 0.01 M 2,4,6-tripyridyl-s-triazine (TPTZ) in hydrochloric acid solution, and 0.02 M ferric chloride at the ratio of 10:1:1 was freshly prepared. Approximately 150 µl of the aliquot extract was mixed with 2850 µl of FRAP working solution and incubated for 30 mins in the dark. A UV-Vis spectrophotometer (VARIAN, Cary 50) was used to compare the absorbance changes of the blue-coloured ferrous-tripyridyltriazine complex at 593 nm. The ascorbic acid (Sigma-Aldrich) was employed as a standard and its calibration curve was used to calculate the FRAP value.

2.5.2 DPPH free radical scavenging activity

The 2,2-Diphenyl-1-picryl-hydrazylhydrate (DPPH) free radical scavenging method is commonly used to measure antioxidant capacity. The assay was performed using a modification of the method decided by Nur Diyana *et al.* (2021). A freshly made 2850 µL of 1,1-diphenyl-2-picryl-2hydrazil (DPPH) methanolic solution was combined with 150 µL extract and vortexed thoroughly. The mixture was incubated in dark conditions for 30 mins at room temperature. The absorbance was read at 515 nm using a UV-Vis spectrophotometer. The ascorbic acid (Sigma-Aldrich) was employed as the standard and its calibration curve was used to calculate the DPPH value.

2.6 Statistical analysis

The mean differences between different samples were analysed using a one-way analysis of variance (ANOVA) followed by Turkey's test at the significance

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level of $p<0.05$. The experiment was complemented with three replicates. The differences between ginger extracts are represented as mean value ± standard deviation (SD) (n = 3). The Minitab software Version 18 (Minitab, LLC) was used to analyse the collected data from this study.

3. Results and discussion

3.1 Determination of total phenolic content

Two different cultivation methods of *Z. officinale* Roscoe using fertigation technology and conventionally slope-grown were examined. The ginger was extracted using three different approaches: a) WE, b) HAE and c) EAE. The total phenolic content in WE, HAE and EAE were described in Figure 1. In both different cultivated ginger extracts, the determined total phenolic content of the EAE extracts (267.481±0.404 and 247.834±1.675 mg GAE/g) were highly significant at $p<0.05$ compared to WE (91.196±0.044 and 122.290±0.153 mg GAE/g) and HAE extracts (83.613±0.044 and 129.847±2.777 mg GAE/g). This is in accordance with a study reported by Nagendra Chari *et al*. (2013), where the total polyphenol content of ginger treated with cellulase enzyme resulted in higher extraction at 37.5 mg/g compared to organic solvent, ethanol and acetone extraction. According to Ghasemzadeh *et al*. (2010b), the polyphenolic compounds are known to have antioxidant activity, and the action of the extracts is most likely attributable to these compounds. In addition, water is the most polar solvent used in extraction because it can dissolve a wide range of polar compounds, inexpensive, safe, non-flammable. However, it encourages microorganisms' growth, which results in a hydrolysis process (Abubakar and Haque, 2020). A study by Mukherjee *et al*. (2014), explained that the increment of extraction temperature enhanced the extraction of ginger extract rate of polyphenol but also can increase the polyphenol oxidation. Whilst, a high-temperature treatment can cause polysaccharides on the cell wall to diffuse to the solvent, weakening the cell wall's integrity (Yang *et al*., 2009).

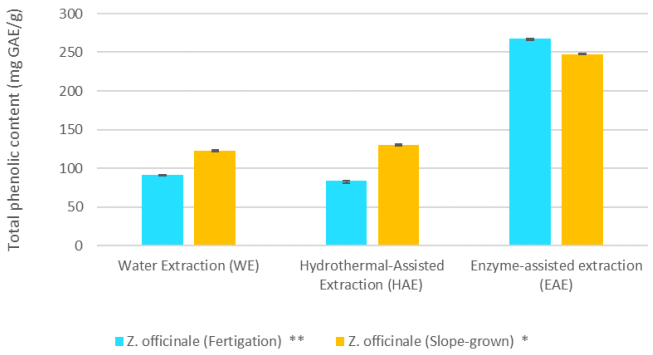

Figure 1. Total phenolic content (TPC) of different *Zingiber officinale* Roscoe using different extraction approaches. Different cultivating methods of ginger showed statistically significant difference at $p<0.05$.
**Fertigation cultivation method: all extracts are significantly different
*Slope grown cultivation method: EA are significantly different with WE and HAE.

3.2 Determination of phenolic acid composition

Phenolic acids are secondary metabolites that seldomly discovered in their natural condition and are found in low concentrations. Plants mostly display phenolic compounds in bonded form, such as glycosides and esters. The amount of phenolic acids and other phenolic compounds in raw plant materials depends on a variety of variables, such as climate, cultivation, fertiliser, and harvest season. In addition, storage condition and methods of extract preparation of plant materials must also crucial (Arceusz *et al.,* 2013). Phenolic acids are regarded as an important type of antioxidant. They can also work through other processes, such as electron donation or singlet oxygen quenching, which are less well-known. The aromatic ring and its substituents influence the stability of various phenolic acids, resulting in different acid stabilities (Tohma *et al*., 2016). Phenolic acids have been shown to offer possible health benefits like antioxidants and anti-inflammatory (Kumar and Goel, 2019). Strong antioxidant sources in ginger can be described by the presence of phenolic acids as tabulated in Table 1. The content of phenolic acids was measured using UPLC and the mass spectra of the sample were compared with authentic compounds as external standard. The EAE extracts from both different cultivation methods of ginger obtained significantly highest ferulic acid at 1.165±0.118 mg/g (fertigation) and 0.610±0.062 mg/g (slope-grown) compared to other extraction methods. EAE extracts of fertigated ginger (0.071±0.001 mg/g syringic acid; 0.056±0.007 mg/g gallic acid; 0.025±0.040 mg/g p-Coumaric acid and 0.023±0.003 mg/g vanillic acid) released more secondary metabolites compared to EAE extracts of slope-grown ginger (0.046±0.006 mg/g syringic acid; 0.049±0.002 mg/g gallic acid; 0.021±0.002 mg/g p-Coumaric acid and 0.020±0.004 mg/g vanillic acid). However, water extracts of both cultivated gingers showed no detection of syringic, p-coumaric and ferulic acids. Only gallic and vanillic acid were detected in fertigated ginger using water extraction at 0.089±0.002 and 0.256±0.021 mg/g, respectively. In addition, HAE extract of fertigated ginger only showed 0.050±0.011 mg/g gallic acid. The HAE extract of slope-grown ginger quantified the presence of 0.011±0.002 mg/g gallic acid, 0.033±0.003 mg/g p-Coumaric acid and 0.020±0.002 mg/g of ferulic acid. A study by Ghasemzadeh *et al*. (2010b), also discovered that phenolic acids such as gallic acid, vanillic acid, ferulic acid, tannic acid, cinnamic acid and

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Table 1. Phenolic acid composition of different cultivation methods of *Zingiber officinale* Roscoe extracts using different extraction approaches.

	Gallic acid (mg/g)	Vanillic acid (mg/g)	Syringic acid (mg/g)	p-Coumaric acid (mg/g)	Ferulic acid (mg/g)
Fertigation *Z. officinale* Roscoe					
Water extraction	0.089±0.002^{a}	0.256±0.021^{a}	ND^{b}	ND^{b}	ND^{b}
Hydrothermal-assisted extraction	0.050±0.011^{b}	ND^{b}	ND^{b}	ND^{b}	ND^{b}
Enzymatic-assisted extraction	0.056±0.007^{b}	0.023±0.003^{b}	0.071±0.001^{a}	0.025±0.04^{a}	1.165±0.118^{a}
Slope-grown *Z. officinale* Roscoe					
Water extraction	ND^{c}	ND^{b}	ND^{b}	ND^{c}	ND^{b}
Hydrothermal-assisted extraction	0.011±0.002^{a}	ND^{b}	ND^{b}	0.033±0.003^{a}	0.020±0.002^{b}
Enzymatic-assisted extraction	0.049±0.002^{b}	0.020±0.004^{a}	0.046±0.006^{a}	0.021±0.002^{b}	0.610±0.062^{a}

Values are presented as mean±SD of triplicates. Values with different superscripts within the same column are statistically significantly different between extracts (p<0.05). Different cultivating methods of ginger showed statistically significant difference at p<0.05. ND: Not detected.

salicylic acid are found in young ginger varieties. Tohma *et al.* (2016) reported higher phenolic compounds found in *Z. officinale* Roscoe using lyophilized water extract with the highest amount of p-Hydroxybenzoic acid followed by p-coumaric acid, pyrogallol, vanillin, ferulic acid, gallic acid, caffeic acid and syringic acid at 321.1, 291.4, 142.4, 101.2, 88.8, 29.8, 9.8 and 0.00 mg/kg compared to both of fertigation and slope grown water extract. However, EAE extract display higher ferulic and syringic acid in comparison with the amounts stated as the cellulose and hemicellulose of ginger are degraded by aid of enzymes, thus releasing these non-extractable polyphenols (Mushtaq *et al*., 2017).

3.3 Determination of antioxidant activities

3.3.1 Ferric reducing antioxidant power

The antioxidant activity of ginger extract from fertigation and slope-grown cultivation systems were determined using the Ferric reducing antioxidant power method that based reduction potential react with potassium ferricyanide (Fe^{3+}) to generate potassium ferrocyanide (Fe^{2+}), which subsequently interacts with ferric chloride to form ferric–ferrous complex with an absorbance maximum at 700 nm (Bhalodia *et al*., 2013). As depicted in Figure 2, the EAE of both cultivated ginger extracts exhibited a significantly high antioxidant activity at 741.867±2.139 and 461.733±0.231 mg AAE/g compared to HAE (410.000±1.200 and 175.467±0.115 mg AAE/g) and WE extract (450.900±0.141 and 125.400±0.283 mg AAE/g) at p>0.05. Plant cell walls comprise a complex array of structural polysaccharides such as cellulose, hemicellulose, pectin, lignin and proteins. This structure gives cells their stability and resistance to intracellular component extraction (Gligor *et al*., 2019). As a result, cellulase enzymes with specialised hydrolytic characteristics are used. This could be explained by the higher hydrolytic capacity of the cellulase and its broad substrate selectivity. In a study conducted by Nagendra Chari *et al*. (2013), cellulase exhibited better cell wall degradation of *Z. officinale* Roscoe. Ghasemzadeh *et al*. (2010a), also reported a higher FRAP value of *Z. officinale* Roscoe rhizome extract at 680.68±18.38 µmol Fe(II)/g dry weight.

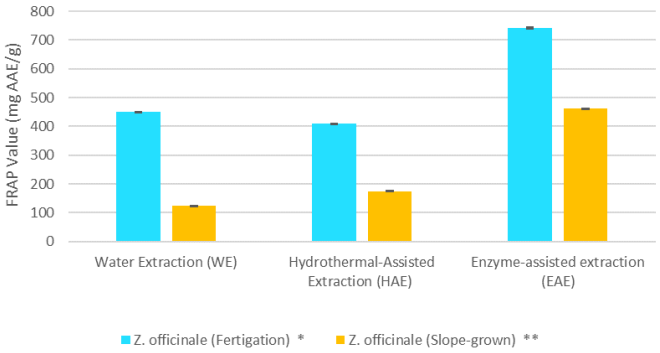

Figure 2. Ferric reducing antioxidant power (FRAP) of different *Zingiber officinale* Roscoe using different extraction approaches. Different cultivating methods of ginger showed statistically significant difference at p<0.05.
**Fertigation cultivation method: all extracts are significantly different
*Slope grown cultivation method: EA are significantly different with WE and HAE.

3.4 DPPH free radical scavenging activity

On the other hand, the antioxidant activity of different cultivated ginger extracts was measured using DPPH free radical scavenging activity method based on electron transfer that produces a violet solution in ethanol. In the presence of an antioxidant molecule, this free radical, which is stable at room temperature, is reduced, yielding a colourless ethanol solution (Huang *et al*., 2005). According to the findings of this study (Figure 3), ginger extracts exhibit strong free radical scavenging activity. Therefore, they can be employed as a radical inhibitor or scavenger, perhaps serving as the main antioxidant. The EAE extracts of both ginger scavenged DPPH radical at 132.317±0.672 and 141.293±0.707 mg

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AAE/g, while WE extract scavenged the lowest DPPH radical at 113.638±0.007 and 72.803±0.025 mg AAE/g of dried ginger. Similar to Mukherjee *et al*. (2014), described ginger extract containing significant phenolic content also obtained strong DPPH radical scavenging activity. The antioxidant activity of ginger is dependent on its total polyphenols content.

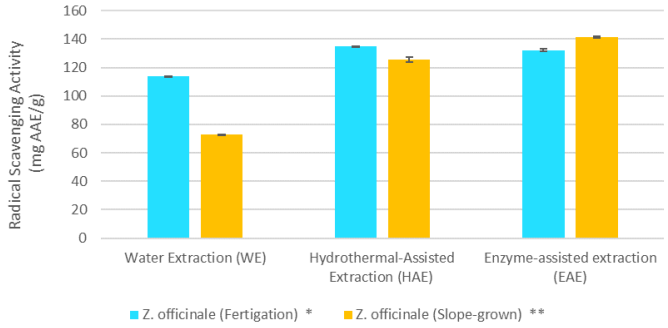

Figure 3. DPPH Free radical scavenging activity of different *Zingiber officinale* Roscoe using different extraction approaches. Different cultivating methods of ginger showed statistically significant difference at $p<0.05$.
**Fertigation cultivation method: all extracts are significantly different
*Slope grown cultivation method: EA are significantly different with WE and HAE.

4. Conclusion

The EAE approach significantly influenced ginger's total phenolic composition and antioxidant activities. The EAE extracts of both fertigation and slope-grown cultivated ginger extracts obtained high ferulic acid, which suggested contributing antioxidant activities of both gingers. The study suggests that enzymatic techniques may offer a greener solution and help add bio -functional value to the ginger extract, as the bioactive compounds and bioactivity values exhibited are value-added products and commercially important.

Conflict of interest

The authors declare no conflict of interest.

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References

Abubakar, A.R. and Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy and BioAllied Sciences,* 12(1), 1-10. https://doi.org/10.4103/jpbs.JPBS_175_19

Bae, J.H., Park., Y.J., Namiesnik, J., Gulcin, L., Kim, T.C., Kim, H.C., Heo, B.G., Gorinstein, S. and Ku, Y.G. (2016). Effects of artificial lightning on bioactivity of sweet red pepper (*Capsicum annuum* L.). *International Journal of Food Science and Technology*, 51(6), 1378-1385. https://doi.org/10.1111/ijfs.13116

Bhalodia, N.R., Nariya, P.B., Acharya, R.N. and Shukla, V.J. (2013). In vitro antioxidant activity of hydro alcoholic extract from the fruit pulp of *Cassia fistula* Linn. *An International Quarterly Journal of Research in Ayurveda*, 34(2), 209-214. https://doi.org/10.4103/0974-8520.119684

Chaves, J.O., de Souza, M.C., da Silva, L.C., Lachos-Perez, D., Torres-Mayanga, P.C., Machado, A.P. F., Forster-Carneiro, T., Vazquez-Espinosa, M., Gonzalez-de-Peredo, A.V., Barbero, G.F. and Rostagno, M.A. (2020). Extraction of flavonoids from natural sources using modern techniques. *Frontier in Chemistry*, 8, 507887. https://doi.org/10.3389/fchem.2020.507887

Chemat, F., Vian, M.A. and Cravotto, G. (2012). Green extraction of natural products: Concept and principles. *International Journal of Molecular Sciences,* 13, 8615-8627. https://doi.org/10.3390/ijms13078615

Chen, S., Xing, X.H., Huang, J.J. and Xu, M.S. (2011). Enzyme-assisted extraction of flavonol transglycosylation catalysed by *Penincilium decumbers* cellulase. *Enzyme and Microbial Technology*, 48(1), 100-105. https://doi.org/10.1016/j.enzmictec.2010.09.017

Department of Statistics Malaysia. Akaun Pembekalan dan Penggunaan Komoditi Pertanian Terpilih, Malaysia (2016-2020). Retrieved on December 1, 2021 from DOSM Website: https://www.dosm.gov.my [In Bahasa Malaysia].

Ghasemzadeh, A., Jaafar, H.Z.E. and Rahmat, A. (2010a). Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia Young Ginger (*Zingiber officinale* Roscoe). *Molecules*, 15, 4324-4333. https://doi.org/10.3390/molecules15064324

Ghasemzadeh, A., Jaafar, H.Z.E. and Rahmat, A. (2010b). Elevated carbon dioxide increases contents of flavonoids and phenolic compounds and antioxidant activities in Malaysia young ginger (*Zingiber officinale* Roscoe.) varieties. *Molecules*, 15, 7907-7922. https://doi.org/10.3390/molecules15117907

Gligor, O., Mocan, A., Moldovan, G., Locatelli, M., Grisan, G. and Ferreira, I.C.F.R. (2019). Enzyme-assisted extraction of polyphenols-A comprehensive review. *Trends in Food Science and Technology,* 88 (5), 302-315. https://doi.org/10.1016/j.tifs.2019.03.029

Grzanna, R., Lindmark, L. and Frondoza, C.G. (2005). Ginger-An herbal medicinal product with broad anti-inflammatory actions. *Journal of Medicinal Food*, 8 (2), 125–132. https://doi.org/10.1089/jmf.2005.8.125

Haida, Z. and Hakiman, M. (2019). A comprehensive review on the determination of enzymatic assay and nonenzymatic antioxidant activities. *Food Science and Nutrition*, 7(5), 1555-1563. https://doi.org/10.1002/fsn3.1012

Huang, D.J., Ou, B.X. and Prior, R.L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry,* 53, 1841-1856. https://doi.org/10.1021/jf030723c

Kumar, N. and Goel, N. (2019). Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnology Reports*, 24, e00370. https://doi.org/10.1016/j.btre.2019.e00370

Li, H., Gao, Y.M., Zhang, J., Wang, L. and Wang, X.X. (2013). Ultra-performance liquid chromatography fingerprinting for quality control of *Phragmitis rhizoma* (Lugen) produced in Baiyangdian. *Pharmacognosy Magazine,* 9(36), 285-289. https://doi.org/10.4103/0973-1296.117810

Mao, Q., Xu, X., Cao, S., Gan, R., Corke, H. and Li., H. (2019). Bioactive compounds and bioactivities of ginger (*Zingiber officinale* Roscoe). *Foods*, 8(6), 185. https://doi.org/10.3390/foods8060185

Marathe, S.J., Jadhav, S.B., Bankar, S.B. and Singhal, R.S. (2017). Enzyme-assisted extraction of bioactives. In Puri, M. (Ed.) Food Bioactives. Extraction and Biotechnology Applications, p. 171-201. Cham, Netherlands: Springer. https://doi.org/10.1007/978-3-319-51639-4_8

Mukherjee, S., Mandal, N. and Mondal, B. (2014). An approach towards optimization of the extraction of polyphenolic antioxidants from ginger (*Zingiber officinale*). *Journal of Food Science and Technology*, 51(11), 3301-3308. https://doi.org/10.1007/s13197-012-0848-z

Mushtaq, M., Sultana, B., Akram, S., Anwar, F., Adnan A., Rizvi, S.S.H. (2017). Enzyme-assisted supercritical fluid extraction: An alternative and green technology for non-extractable polyphenols. *Analytical and Bioanalytical Chemistry*, 409, 3645-3655. https://doi.org/10.1007/s13197-012-0848-z

Nagendra chari, K.L., Manasa, D., Srinivas, P. and Sowbhagya, H. B. (2013). Enzyme-assisted extraction of bioactive compounds from ginger (*Zingiber officinale* Roscoe). *Food Chemistry,* 139(1-4), 509-514. https://doi.org/10.1016/j.foodchem.2013.01.099

Nawaz, H., Shad, M.A., Rehman, N., Andaleeb, H. and Ullah, N. (2020). Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. *Brazilian Journal of Pharmaceutical Sciences,* 56, e17129. https://doi.org/10.1590/s2175-97902019000417129

Nur Diyana, A., Puteh, F., Mohd Zulkifli, N.I. and Hasnan, N.Z.N. (2021). Evaluation of total phenolic content and antioxidant activities from different extraction techniques of *Helianthus tuberosus*. *Advances in Agricultural and Food Research Journal*, 2(1), a0000165.

Prasad, S. and Tyagi, A.K. (2015). Ginger and Its Constituents: Role in Prevention and Treatment of Gastrointestinal Cancer. *Gastroenterology Research and Practice,* 2015, 142979. https://doi.org/10.1155/2015/142979

Shinde, R.R. and Shinde, N.H. (2017). Extraction of Caffeine from Coffee and preparation of Anacin drug. *International Journal of Engineering Research and Technology,* 10(1), 236-239.

Stoilova, I., Krastanov, A., Stoyanova, A., Denev, P. and Gargova, S. (2007). Antioxidant activity of a ginger extract (*Zingiber officinale*). *Food Chemistry*, 102(3), 764-770. https://doi.org/10.1016/j.foodchem.2006.06.023

Tohma, H., Gulcin, I., Bursal, E., Goren, A.C., Alwasel, S.H. and Koksal. (2016). Antioxidant activity and phenolic compounds of ginger (*Zingiber officinale* Rosc.) determined by HPLC-MS/MS. *Food Measure,* 11, 556–566. https://doi.org/10.1007/s11694-016-9423-z

Truong, D., Nguyen, D.H., Ta, N.T.A., Bui, A.V., Do, T.H. and Nguyen, H.C. (2019). Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidants, and In Vitro Anti-Inflammatory Activities of *Severinia buxifolia. Journal of Food Quality,* 2019, 8178294. https://doi.org/10.1155/2019/8178294

Tyler, V.E. (1993). The Therapeutic Use of Phytomedicinals. New York, USA: Pharmaceutical Products Press.

Yang, L., Jiang, J., Li, W., Chen, J., Wang, D. and Zhu, L. (2009). Optimum extraction process of polyphenols from the bark of *Phyllanthus emblica* L. based on the response surface methodology. *Journal of Separation Science*, 32(9), 1437–1444. https://doi.org/10.1002/jssc.200800744

Xu, G., Ye, X., Chen, J. and Liu, D. (2007). Effect of heat treatment on the phenolic compounds and antioxidant capacity of citrus peel extract. *Journal of Agricultural and Food Chemistry,* 55, 330-335. https://doi.org/10.1021/jf062517l

