The effect of ginger (*Zingiber officinale*) phytochemicals on the bioaccessibility of iron (Fe) and zinc (Zn) in an in-vitro digestive system model

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Abstract. Iron deficiency anaemia remains a major nutritional problem among adolescent girls due to low iron bioaccessibility from dietary sources. This study examined the effects of phytochemicals in ginger (*Zingiber officinale*) on iron (Fe) and zinc (Zn) bioaccessibility using an in-vitro digestive system model. The samples consisted of elephant ginger rhizome (*Z. officinale var. officinarum*), red ginger (*Z. officinale var. rubrum*), and emprit ginger (*Z. officinale var. amarum*). A randomized block design was applied with extraction temperatures of 40, 50, and 60°C, each in three replications. Ginger extracts were prepared by heated maceration for five hours and analysed for total phenolic content, total flavonoids, 6-gingerol, and quercetin. Based on phytochemical characteristics, three representative extracts (sm1, sm2, and sm3) were selected. Differences among extract types were evaluated using analysis of variance (ANOVA). The selected extracts (200 µg/mL) were combined with a multiple micronutrient supplement (MMS) and subjected to INFOGEST 2.0 in vitro digestion to determine Fe and Zn bioaccessibility. The results indicated that ginger phytochemical profiles affected soluble Fe and Zn concentrations in digesta, with Fe ranging from 0.0400–0.0454 mg/mL and Zn from 0.3360–0.4403 µg/mL. These findings support ginger phytochemicals as mineral bioaccessibility modulators for functional food strategies against iron deficiency anaemia.

1 Introduction

Iron (Fe) and zinc (Zn) deficiencies remain major global health concerns, particularly among children, adolescents, and pregnant women. Iron deficiency can lead to anaemia, resulting in cognitive impairment, reduced productivity, and increased maternal and child morbidity and mortality [1]. In Indonesia, ferritin deficiency affects 34.2% of children and contributes to the risk of iron-deficiency anaemia, while zinc deficiency is associated with impaired growth, reduced immune function, and increased infection risk [2, 3].

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Despite widespread supplementation and fortification efforts, Fe and Zn bioavailability remains a challenge [4]. Ginger (*Zingiber officinale*) contains bioactive phytochemicals, including gingerol, shogaol, paradol, and quercetin which may modulate mineral bioaccessibility during digestion [5]. In particular, flavonoids such as quercetin have been reported to enhance iron metabolism through chelation and antioxidant mechanisms, suggesting the potential of ginger phytochemicals to improve mineral bioaccessibility and anaemia-related biomarkers [6].

In vitro digestion models are widely used to evaluate mineral bioaccessibility under simulated physiological conditions and to investigate bioactive–mineral interactions in food matrices [7]. However, limited information is available regarding the effects of ginger phytochemicals on the bioaccessibility of both Fe and Zn, particularly within the standardized INFOGEST 2.0 model and in relation to ginger variety and extraction conditions. Therefore, this study aimed to evaluate the impact of ginger phytochemicals on Fe and Zn bioaccessibility using an in vitro digestion model, providing insights for the development of food-based strategies and functional foods to improve essential mineral bioaccessibility and potentially reduce the risk of anaemia.

2 Materials and methods

2.1 Research design

This study employed a randomized complete block design (RCBD) with three replications. The ginger varieties utilized were elephant ginger (*Zingiber officinale* var. officinarum), red ginger (*Zingiber officinale* var. rubrum), and emprit ginger (*Zingiber officinale* var. amarum). The extraction process was conducted using 70% ethanol as a solvent, with heat treatment applied at three distinct temperatures (40, 50, and 60°C).

2.2 Plant material and preparation of ginger extract

Elephant, red, and emprit ginger were obtained from *Kelompok Wanita Tani* (KWT) Tani Mukti I and KWT Kemuning Jaya in Sukabumi, Indonesia. Elephant and emprit ginger were harvested at six months of age, while red ginger was harvested at eight months. A multivitamin and mineral supplement (MMS; Vitamin Angels), containing 30 mg Fe and 15 mg Zn, was used as a standardized micronutrient source to evaluate the effects of ginger phytochemicals on mineral bioaccessibility, with MMS alone serving as the control.

Three ginger varieties (elephant, red, and emprit) were extracted by warm maceration in 70% ethanol at 40°C, 50°C, or 60°C for 2 h, yielding nine crude extracts [5]. After phytochemical characterization (total phenolics, flavonoids, quercetin, and 6-gingerol), the three extracts with the most favourable profiles were combined with MMS and evaluated for Fe and Zn bioaccessibility using an in vitro digestion model.

2.3 Quantification of bioactive potential (total phenol and total flavonoid) and identification of 6-gingerol and quercetin

The total phenolic content was analysed using colorimetric analysis with the Folin-Ciocalteu method, as described by Ali et al. [8] with slight modifications. The absorbance of the samples was measured at a wavelength of 765 nm. Total flavonoid content was determined using a colorimetric method involving aluminium chloride ($AlCl_3$). The analysis was conducted at a wavelength of 437 nm based on Ali et al. [8] with minor modifications.

The 6-gingerol and quercetin content were analysed using an HPLC UV-Vis device: Shimadzu Prominence HPLC system (Shimadzu Corporation, Kyoto, Japan) with a DGU-20A5R module, LC-20AD pump, and SPD-20A detector. The 6-gingerol method referenced Cho et al. [9] with slight modifications. Sample separation was performed using a C18 column (250 mm × 4.6 mm, 5 µm) at 25°C, flow rate of 1.0 mL/min, and detection at 280 nm. The mobile phase was acetonitrile and 1% acetic acid (48:52). The quercetin method referenced Ang et al. [10] with slight modifications. Separation used a C18 column (250 mm × 4.6 mm, 5 µm) at 25°C, flow rate of 1.0 mL/min, and detection at 370 nm. The mobile phase was methanol and 0.4% phosphoric acid (49:51).

2.4 In-vitro bioaccessibility test

The INFOGEST 2.0 method was used to assess mineral bioaccessibility in vitro by simulating oral, gastric, and intestinal digestion. Following the intestinal phase, the digestate was centrifuged, and minerals in the supernatant were quantified by Atomic Absorption Spectroscopy (AAS) after wet ashing.

2.5 Data processing and analysis

Statistical analyses were performed using SPSS for Windows, version 26 (IBM, Armonk, NY, USA). Descriptive data are reported as mean and standard deviation, except 6-gingerol data, which were analysed once per sample. Data underwent one-way ANOVA and Duncan's multiple range test to assess group differences.

3 Results and discussion

3.1 Phytochemical content of ginger extract

The phytochemical composition of ginger extract varies and is influenced by the ginger variety and extraction method used. Analytical measurements have verified the presence of phenolic compounds, flavonoids, 6-gingerol, and quercetin in ginger extract. Table 1 presents the phytochemical profiles of the three selected ginger extracts, which demonstrate the most favourable characteristics among the nine extracts generated in this study. Sm3 had the highest total phenol concentration (268.13±15.41 mg GAE/g), significantly higher ($p \leq 0.05$) than Sm1 (216.07±4.61 mg GAE/g) and Sm2 (194.50±15.15 mg GAE/g), representing a 23.9% increase over the lowest. Total flavonoid content showed no significant differences, with concentrations ranging from 78.48±36.74 mg QE/g (Sm1) to 108.79±22.88 mg QE/g (Sm3), representing a modest 38.7% variation. The marker compound 6-gingerol decreased from Sm1 (20.10 mg/g) to Sm2 (15.04 mg/g) to Sm3 (12.13 mg/g). Quercetin was found in Sm1 (16.66±0.21 mg/g) and Sm2 (16.45±0.08 mg/g) with no significant differences, but not in Sm3. Sm3 had superior total phenolic content, while Sm1 had the highest 6-gingerol levels and was among the only varieties with quercetin, suggesting complementary profiles across varieties.

The findings of this study confirmed the presence of various phytochemicals in ginger extracts, including quercetin, total phenolic compounds, and 6-gingerol. The consistent detection of 6-gingerol across different ginger varieties and extraction methods further supports its recognition as a characteristic bioactive compound of ginger [11]. Moreover, the observed variations in quercetin and total phenolic content among Sm1, Sm2, and Sm3 (Table 1) may contribute to differences in iron and zinc bioaccessibility during in-vitro

digestion, given the known involvement of phenolic compounds in chelation and antioxidant mechanisms that potentially affect mineral availability [6].

Table 1. Phytochemical composition in selected ginger extract for mineral bioaccessibility assessment.

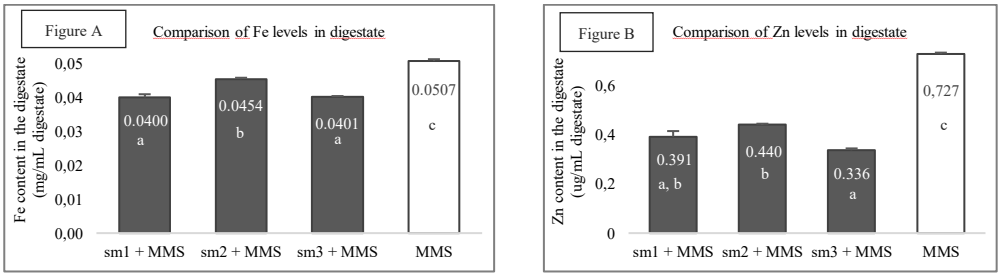
No.	Parameter	Sm1 (Mean±sd)	Sm2 (Mean±sd)	Sm3 (Mean±sd)
1.	Total phenol (mg GAE/g extract)	216.07±4.61[a]	194.50±15.15[a]	268.13±15.41[b]
2.	Total flavonoid (mg QE/g extract)	78.48±36.74[a]	105.76±18.92[a]	108.79±22.88[a]
3.	6-gingerol (mg/g extract)	20.10	15.04	12.13
4.	Quercetin (mg/g extract)	16.66±0.21[a]	16.45±0.08[a]	ND

Note: Sm1=Elephant ginger extract (T=60ºC; concentration=200 µg/mL); Sm2=Red ginger extract (T=60ºC; concentration=200 µg/mL); Sm3=Emprit ginger extract (T=60ºC; concentration=200 µg/mL); ND=Not Detected; [a, b]=Means that do not share the same letter in a row are significantly different at p≤0.05, as determined by the results of ANOVA and Tukey HSD post hoc test

3.2 Bioaccessibility of iron (Fe) and zinc (Zn) from digestate based on INFOGEST 2.0

The three selected ginger extracts (Sm1, Sm2, and Sm3), chosen based on their superior phytochemical profiles among the nine extracts evaluated, were subjected to the INFOGEST 2.0 digestion assay at a concentration of 200 µg/mL in a simulated gastrointestinal system containing multivitamin-mineral supplements (MMS; Vitamin Angels).

Figure 1 presents digestate Fe and Zn concentrations following the INFOGEST 2.0 assay, indicating that ginger phytochemicals influenced mineral release during digestion, with Fe and Zn levels ranging from 0.0400–0.0454 mg/mL and 0.3360–0.4403 µg/mL, respectively.



Note: Sm1=Elephant ginger extract (T=60ºC; concentration=200 µg/mL); Sm2=Red ginger extract (T=60ºC; concentration=200 µg/mL); Sm3=Emprit ginger extract (T=60ºC; concentration=200 µg/mL); MMS=Multivitamin Mineral Supplement (1 tablet=467 mg)
[a, b]=Means that do not share the same letter are significantly different at p≤0.05, as determined by the results of ANOVA and Tukey HSD post hoc test.

Fig. 1. Comparison of iron (Fe) levels (Figure A) and zinc (Zn) levels (Figure B) in the digestate from selected ginger extract mixtures and MMS tablets

Based on the final digestate volume of 50 mL and the Fe and Zn contents in the MMS (30 mg and 15 mg, respectively), these data corresponded to bioaccessibility values of 6.7–7.6% for Fe and 0.11–0.15% for Zn. Statistical analysis of these mineral concentrations using one-way ANOVA followed by Tukey's HSD post hoc test revealed that means not sharing the same letter designation (a, b) were significantly different at p≤0.05. This variation in dissolved Fe and Zn levels among ginger extract and supplement combinations suggests that ginger phytochemical compositions influence the bioaccessible fraction of these minerals, highlighting the importance of characterizing the phytochemical profile in developing ginger-based products to optimize micronutrient bioaccessibility.

Ginger phytochemicals, particularly phenolic and flavonoid compounds such as 6-gingerol and quercetin (Table 1), may enhance Fe bioaccessibility by forming soluble metal complexes and maintaining iron in the more absorbable ferrous (Fe^{2+}) form through their antioxidant activity [6,12]. Similar effects have been reported for flavonoid-rich food matrices, where mineral absorption is influenced by phytochemical composition and concentration [13].

Given the concern regarding iron deficiency in adolescent girls, developing ginger-based functional foods with standardized phytochemical profiles offers a relevant non-pharmacological intervention strategy. Based on these in vitro findings, ginger extracts with characterized phytochemical profiles warrant further investigation as potential functional food components for applications aimed at improving the bioaccessibility of essential minerals. However, the in vitro scope of this study does not establish direct evidence of efficacy in preventing iron deficiency anaemia in human populations.

4 Conclusion

Warm maceration of ginger yielded extracts with varied phytochemical traits across varieties. HPLC showed quercetin and [6]-gingerol as main components. Standardized ginger extracts may improve mineral bioaccessibility. The phytochemicals in ginger, such as phenolic compounds and 6-gingerol, can modulate the soluble concentrations of Fe and Zn, demonstrating potential for use in functional foods to enhance essential mineral absorption. Despite the presence of dissolved mineral concentrations, the overall bioaccessibility remained low (6.7–7.6% for Fe and 0.11–0.15% for Zn), indicating that despite phytochemical modulation, the majority of minerals remained in forms unavailable for absorption under in vitro conditions. This finding underscores the complexity of mineral bioavailability and suggests that formulation optimization is essential before functional food development. However, the study's scope is limited to in-vitro bioaccessibility modelling and does not directly assess clinical efficacy in addressing iron deficiency anaemia in human subjects.

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