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Comparison of the Antioxidant Activity of Malaysian Ginger (*Zingiber officinale* Roscoe) Extracts with that of Selected Natural Products and its Effect on the Viability of Myoblast Cells in Culture

(Perbandingan Aktiviti Antioksidan Halia (*Zingiber officinale* Roscoe) Malaysia dengan Produk Semula Jadi Terpilih dan Kesannya terhadap Kebolehidupan Sel Mioblas dalam Kultur

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ABSTRACT

Ginger has been proven to possess various therapeutic effects, including antibacterial, anticancer, anti-inflammatory, and antioxidant effects. However, data on the comparison of ginger antioxidant activity with that of other natural products are still lacking. This study aimed to analyse and compare the antioxidant properties of two types of Malaysian ginger extracts (GE1 and GE2) with that of selected natural products. The antioxidant activities were measured by 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and ferric reducing antioxidant power (FRAP) assays, while cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfonyl)-2H-tetrazolium (MTS) assay. The order of the DPPH scavenging activities was as follows: vitamin C > palm tocotrienol-rich fraction (TRF) > α-tocopherol > N-acetylcysteine (NAC) > Ficus deltoidea *> butylated hydroxytoluene (BHT) >* Centella asiatica *> GE2 > GE1 >* Moringa oleifera *>* Kelulut *honey; the order of the mean FRAP value was as follows: NAC > α-tocopherol > BHT > TRF >* Ficus deltoidea *>* Moringa oleifera *> GE2 = GE1 >* Centella asiatica *>* Kelulut *honey. The viability assays showed that both ginger extracts significantly increased the percentage of viable cells ($p < 0.05$). In conclusion, neither of the ginger extracts was cytotoxic toward cells and both possessed comparable antioxidant properties, indicating their potential for ameliorating oxidative stress.*

Keywords: Antioxidant property; gingerol; Malaysian ginger; myoblasts; shogaol

ABSTRAK

Halia telah terbukti mempunyai pelbagai kesan terapeutik termasuklah kesan anti-bakteria, anti-kanser, anti-radang dan antioksidan. Namun sehingga kini, perbandingan aktiviti antioksidan antara halia dengan produk semula jadi yang lain masih lagi kurang. Oleh itu, tujuan kajian ini dijalankan adalah untuk menganalisis dan membandingkan ciri-ciri antioksidan yang ada pada dua jenis ekstrak halia (GE1 dan GE2) yang ada di Malaysia dengan produk semula jadi yang lain. Aktiviti antioksidan telah diukur melalui asai 2,2-difenil-1-fikril-hidrazil-hidra (DPPH) dan asai aktiviti penurunan kuasa antioksidan ion ferik (FRAP), manakala kebolehidupan sel ditentukan melalui asai 3-(4,5-dimetiltiazol-2-yil)-5-(3-karboksimetoksifenil)-2-(4-sulfonil)-2H-tetrazolium (MTS). Susunan untuk aktiviti hapus sisa radikal bebas DPPH adalah seperti berikut: vitamin C > fraksi kaya tokotrienol (TRF) > α-tokoferol > NAC > Ficus deltoidea *> BHT >* Centella asiatica *> GE2 > GE1 >* Moringa oleifera *> madu Kelulut, manakala susunan untuk aktiviti penurunan kuasa antioksidan ion ferik (FRAP) adalah seperti berikut: NAC > α-tokoferol > BHT > TRF >* Ficus deltoidea *>* Moringa oleifera *> GE2 = GE1 >* Centella asiatica *> madu Kelulut. Asai kebolehidupan sel menunjukkan peratus kebolehidupan sel yang dirawat dengan kedua-dua ekstrak halia meningkat secara signifikan ($p < 0.05$). Kesimpulannya, kedua-dua jenis ekstrak halia ini tidak memberi kesan toksik terhadap sel dan mengandungi ciri-ciri antioksidan yang berpotensi mengurangkan aras tekanan oksidatif.*

Kata kunci: Ciri antioksidan; gingerol; halia Malaysia; mioblas; shogaol

Introduction

Ginger (*Zingiber officinale*) is a medicinal plant that is derived from one of two species, *Zingiber officinale* Roscoe and *Zingiber officinale* Rubrum (Ahmed et al. 2011; Shimoda et al. 2010). *Zingiber officinale* Roscoe belongs to the family Zingiberaceae, which can be found in subtropical and tropical Asia, Africa, Far East Asia, China, and India (Tanaka et al. 2015). Several bioactive components have been identified in this ginger species, such as 6-gingerol, 6-shogaol, 10-gingerol, gingerdiones, gingerdiols, paradols, 6-dehydrogingerols, 5-acetoxy-6-gingerol, 3,5-diacetoxy-6-gingerdioal and 12-gingerol, which contribute to the many biological activities of ginger (Park et al. 2008; Tanaka et al. 2015; Van Breemen et al. 2011). The most abundant active compounds found in fresh ginger are 6-gingerol and 6-shagoal (Jolad et al. 2004; Tanaka et al. 2015).

The bioactive compounds and constituents of ginger have various therapeutic effects, including antibacterial (Chakotiya et al. 2017; Park et al. 2008; Valera et al. 2015) anticancer (Habib et al. 2008; Pashaei-Asl et al. 2017; Saha et al. 2014), anti-inflammatory (Ezzat et al. 2018; Habib et al. 2008), antidiabetic (Al-Amin et al. 2006), gastroprotective (Liju et al. 2015), antioxidant (Shirin-Adel & Prakash 2010; Si et al. 2018), and neuroprotective (Hussein et al. 2017) effects. The antioxidant property of *Zingiber officinale* Roscoe has been proven to reduce many oxidative stress-related medical conditions, including diabetes, cardiovascular disease (Wang et al. 2017), cancer (Yasmin Anum et al. 2008) and inflammatory disease (Mozaffari-Khosravi et al. 2016). A previous study showed that ginger extract displays strong antioxidant properties and can perform a function similar to that of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), which eliminate superoxide radicals and hydrogen peroxide that can cause oxidative damage to cells (Ahmad et al. 2006). Mahluji et al. (2013) demonstrated that the antioxidant properties of ginger had a positive effect on insulin resistance in type 2 diabetes patients by increasing glucose tolerance and uptake in the body, which caused a decrease in insulin resistance. In addition to its antioxidant properties, ginger also acts synergistically with anti-tuberculosis treatments by decreasing tumour necrosis factor alpha (TNF-α), lipid peroxidation and malondialdehyde (MDA) in tuberculosis patients (Kulkarni & Deshpande 2016).

Antioxidant agents play a crucial role in scavenging free radicals and reactive oxygen species (ROS), which are produced by normal biological processes in the body or from exogenous sources (Waris & Ahsan 2006). The excessive production of ROS contributes to the oxidation of biological molecules such as carbohydrates, DNA, proteins and lipids, resulting in the failure of biological function as well as cellular damage by apoptosis, which later contributes to disease progression (Ramalingam & Kim 2012; Suzuki et al. 2015). High production of ROS also results in activation of transcriptional factors and kinase enzymes, which can cause an imbalance in protein synthesis and breakdown (Meng & Yu 2010). The antioxidant properties of ginger are influenced by the extraction and processing method. A study conducted by Rigane et al. (2018) found that methanol extract exhibited a higher anti-radical capacity than ethyl acetate extract. This was in accordance with another study that reported that ethanol extraction of ginger resulted in better antioxidant properties than the water extraction method (Tohma et al. 2017).

In addition to ginger, there are other natural products that have been reported to act as potent antioxidants, including honey, *Moringa oleifera*, *Ficus deltoidea*, and *Centella asiatica*. A previous study showed that *Moringa oleifera,* which possesses high antioxidant capacity, has the potential to prevent disease caused by oxidative stress (Wright et al. 2017). *Moringa oleifera* has been shown to possess higher reducing power and to lower free radicals better than five selected vegetables, cabbage, spinach, broccoli, cauliflower, and peas (Pakade et al. 2013). A study performed by Abrahim et al. (2018) showed that *Ficus deltoidea* also has excellent antioxidant activity without showing any cytotoxic effect on normal liver cells (Abrahim et al. 2018; Misbah et al. 2013). A study on another natural product, *Centella asiatica,* showed that different extraction solvents demonstrated various degrees of antioxidant potential, which contributed to the differences in pharmacological activity (Rahman et al. 2013). Another natural product, *Tualang* honey, exhibited the most effective free-radical scavenging activity compared to other types of honey (Kishore et al. 2011). The antioxidant properties of these natural products were reported to be due to the existence of their phenolic content (Abrahim et al. 2018; Ariffin et al. 2011; Kishore et al. 2011; Misbah et al. 2013; Pourreza 2013).

Data on the comparison of the antioxidant properties of ginger with those of other natural products, however, are still lacking. Therefore, the present study aimed to analyse and compare the antioxidant properties of two types of Malaysian ginger extracts with the antioxidant properties of other selected natural products, such as *Kelulut* honey, *Moringa oleifera*, *Ficus deltoidea*, *Centella asiatica*, tocopherol, and tocotrienol-rich fraction (TRF). The effects of ginger extracts on the viability of myoblast cells in culture were also studied.

Materials and Methods

Ginger extract preparation

Ginger (*Zingiber officinale* Roscoe) extract was processed by using subcritical water extraction and obtained from Universiti Teknologi Malaysia (UTM, Malaysia). In this study, the extraction procedure was carried out on dried ginger by using two types of extraction methods. For the extraction of ginger extract 1 (GE1), the optimum processing conditions were 130 °C for 30 min, while the solvent-to-solid ratio was 28/2 $mLmg^{-1}$. In the extraction of ginger extract 2 (GE2), the optimum conditions were 120 °C for 20 min, and the solvent-to-solid ratio was 28/2 $mLmg^{-1}$. Thus, two types of ginger extracts (GE1 and GE2) were produced and further analysed in this study.

Instrumentation and chromatographic conditions

The analytical method for the detection of 6-gingerol and 6-shogaol was performed by using a Waters Alliance 2695 LC system (USA) connected with a Waters model 2996 photodiode-array detector (USA). Data were collected and processed with the Empower workstation. The optimum UHPLC system consisted of a C_{18} reversed-phase column. Gradient elution was performed with water and acetonitrile at a flow rate of 0.4 $mLmin^{-1}$, and photodiode array (PDA) detection was conducted at 282 nm. Water and 100% acetonitrile were used as mobile phase A and mobile phase B, respectively. The separation of the active components of ginger was carried out based on the gradient elution program with water and acetonitrile (Merck, Germany) as follows: from 0 to 15 min, the volumetric ratio was 70:30; from 15 to 16 min, the volumetric ratio was 5.0:95; from 16 to 17 min, the volumetric ratio was 5.0:95 min; from 17 to 20 min, the volumetric ratio was 70:30.

Preparation of natural products for the DPPH and FRAP assays

Stock solutions of *Kelulut* honey (Droness, Kelantan, Malaysia), *Moringa oleifera* (Klau Valley, Integrated Farm Sdn. Bhd. Malaysia), standardised extract of *Ficus deltoidea* (HERbagus Trading, Malaysia), standardised extract of *Centella asiatica* (Forest Research Institute, Malaysia), α-tocopherol (98.9% pure d-ATF, ChromaDex, USA) and TRF (Sime Darby Bhd., Malaysia) were prepared at a concentration of $mgmL^{-1}$. Each natural product was weighed and diluted with ultrapure water or ethanol based on its solubility characteristics. Vitamin C (99% pure L-ascorbic acid, Sigma, USA), NAC (>99% pure *N*-acetyl-L-cysteine, Sigma, USA) and BHT (99% pure 2,6-di-tert-butyl-4-methylphenol, Sigma, USA) were used as controls. The stock solutions of vitamin C, N-acetylcysteine (NAC) and butylated hydroxytoluene (BHT) were prepared by weighing the materials and diluting them with ultrapure water, 100% ethanol and 95% ethanol, respectively, at a concentration of 10 mg/mL. All the controls were used for the quantification of the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging activity.

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging activity

The stock solution of 1,1-diphenyl-2-picryl-hydrazyl (Sigma, USA) was prepared in methanol by mixing 60 mL methanol (Merck, Germany) with 40 mL acetate buffer (Merck, Germany) (pH 5.5). The ginger extracts GE1 and GE2, the natural product extracts and the controls were diluted at a series of final concentrations of 0, 10, 20, 50, 100, 200, 500, and 1000 μgmL^{-1} with the respective diluents. Then, 0.75 mL of the diluted extract or control was mixed with 1.5 mL of 0.009 $mgmL^{-1}$ DPPH in methanol by using a vortex. The mixture was incubated at room temperature for 10 min. Then, the absorbance was recorded at a wavelength of 517 nm with an Enspire Multimode Plate Reader (Perkin Elmer, Singapore). Methanol was used as a control (A_c). The inhibition percentage was calculated based on the following equation:

$$\% \text{ Inhibition} = \frac{(Ac - As)}{Ac} \times 100\% \quad \text{(Garcia et al. 2012)}$$

Ferric reducing antioxidant power (FRAP) assay

The FRAP reagent was prepared by mixing 2.5 mL of 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) solution (Sigma, USA) (10 mM), 2.5 mL ferric chloride hexahydrate ($FeCl_3.6H_2$) solution (Merck, USA) (20 mM) and acetate buffer (Merck, Germany) (300 mM; pH3.6) at a ratio of 1:1:30. Forty microlitres of ginger extract, natural product extract or control at a final concentration of 0, 10, 20, 50, 100, 200, 500, or 1000 μgmL^{-1} was mixed with 1.2 mL of FRAP reagent. The solution was incubated for 10 min. The absorbance was measured at 593 nm by a Enspire Multimode Plate Reader (Perkin Elmer, Singapore). The antioxidant potential of the sample was determined by using a standard curve of ferrous sulphate ($FeSO_4.7H_2O$) (Sigma, USA).

PREPARATION OF MYOBLAST CELLS IN CULTURE

Primary human myoblast cells were purchased from Lonza (Walkersville, MD, USA) and cultured in complete culture media (CCM), skeletal muscle basal medium supplemented with human epidermal growth factor, foetal bovine serum, dexamethasone, L-glutamine, and gentamicin sulphate/amphotericin B (Lonza, MD, USA). Cells were cultured at 37 °C in a humid atmosphere containing 5% CO_2. The cells then underwent serial passaging until they reached the senescence stage. For each passage, the number of population doublings (PD) was calculated as log (N/n)/log 2, where N indicates the number of cells at the time of passaging and n was the number of cells at the seeding stage. The cells were divided into three groups: the young group (PD < 15), presenescent group (15< PD> 20) and senescent group (PD > 20) (Tan et al. 2021).

MTS ASSAY FOR THE DETERMINATION OF CELL VIABILITY

Cell viability was determined by a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfonyl)-2H-tetrazolium (MTS) calorimetric assay. The stock solutions of the ginger extracts GE1 and GE2 were freshly prepared in water at a concentration of 10 $mgmL^{-1}$. The stock solutions were kept for no more than one month at -20 °C. Both ginger extracts were serially diluted with complete culture medium (CCM) to concentrations of 0, 10, 20, 30, 50, 100, 200, 300, 500, and 1000 μgmL^{-1}. Myoblast cells were plated at a density of 2 x 10^4 in 96-well plates before incubation overnight. The medium was then exchanged with the new medium, which contained different concentrations of GE1 or GE2, and the cells were incubated in 5% CO_2 at 37 °C for 24 h. After 24 h of incubation, the media containing either GE1 or GE2 were replaced with new CCM prior to the MTS assay. Then, 20 µL MTS reagent (Promega, USA) was added, and the plate was incubated for another 2 h. The value of the absorbance of MTS was measured at 490 nm by an Enspire Multimode Plate Reader (Perkin Elmer, Singapore).

STATISTICAL ANALYSIS

Each experiment was performed three times, and the data were recorded as the mean ± standard deviation. The significant differences in the DPPH assay, FRAP assay and cell viability assay were analysed by one-way ANOVA. Comparisons between groups were made by a post hoc Tukey test. The analysis was carried out by using SPSS software version 20, and $p < 0.05$ was considered to indicate a significant difference.

RESULTS AND DISCUSSION

ANTIOXIDANT PROPERTIES

From the ultrahigh-performance liquid chromatography (UHPLC) results, both GE1 and GE2 were found to contain 6-gingerol and 6-shogaol as active compounds (Table 1). Figure 1 shows the DPPH scavenging activities of the ginger extracts GE1 and GE2 and the other natural product extracts. The DPPH radical scavenging activity increased with increasing concentrations of all extracts. The order of the DPPH scavenging activities was as follows: Vitamin C > TRF > α-Tocopherol > NAC > *Ficus deltoidea* > BHT > *Centella asiatica* > GE2 > GE1 > *Moringa oleifera* > *Kelulut* honey. Interestingly, as shown in Figure 1, there was a significant difference in the DPPH scavenging activities between GE1 and GE2 at concentrations of 500 µg/mL and 1000 μgmL^{-1} ($p < 0.05$).

Figure 2 illustrates the FRAP activities of GE1, GE2 and the other natural product extracts. The FRAP activities increased with increasing concentrations of all the extracts. The order of the mean FRAP activity value was as follows: NAC > α-Tocopherol > BHT > TRF > *Ficus deltoidea* > *Moringa oleifera* > GE2 = GE1 > *Centella asiatica* > *Kelulut* honey. However, no significant difference was observed in the FRAP activities between GE 1 and GE 2 at all concentrations used in the study.

In this study, DPPH scavenging activity assays and FRAP activity assays were performed to evaluate the antioxidant properties of 6-gingerol and 6-shogaol in these two types of Malaysian ginger extracts as well as those of other active compounds in the selected natural product extracts. In the DPPH assay, the antioxidant agent present in the two bioactive components (gingerol and shogoal) and in the other studied extracts scavenged the DPPH free radical by donating hydrogen, which contributed to the formation of the nonradical form of DPPH (Kedare & Singh 2011). The DPPH free radical is an organic nitrogen free radical that is characterised by a deep purple colour. Therefore, during the assay, the colour changed from purple to yellow. However, the FRAP assay was performed based on the reducing power of the antioxidants in the studied compounds. The determined antioxidant property reduced the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}), which resulted in the formation of a blue complex (Fe^{2+}/TPTZ) (Gupta 2015). The increase in FRAP activity indicated an increase in antioxidant capacity, as the FRAP assay result was based on the reduction of ferric

ions. This is because antioxidants are reducing agents that can donate a single electron or hydrogen for reduction.

Based on the findings of this study, both types of Malaysian ginger extracts, which contain both gingerol and shogaol, exhibited higher levels of antioxidant activity than *Moringa oleifera* and *Kelulut* honey but lower levels of antioxidant activity than vitamin C, NAC, TRF, α-tocopherol, *Ficus deltoidea*, BHT, and *Centella asiatica*. A previous study carried out by Nadeem et al. (2012) reported that ginger exhibited higher antioxidant activity than cumin extract, which was correlated with its total phenolic content. Another study performed by El-Ghorab et al. (2010) reported that in DPPH and FRAP assays, cumin essential oil displayed the highest antioxidant activity, followed by dried ginger essential oil and fresh ginger essential oil. Ginger also showed higher DPPH and FRAP activities than turmeric (Maizura et al. 2011). However, Misbah et al. (2013) found that the combination of ginger and turmeric powder showed higher free radical scavenging activity than the individual extracts of ginger and turmeric powder. In another study, ginger extract demonstrated better antioxidant properties than garlic, onion, thyme, aloe vera, mint, and oak (Abdul Qadir et al. 2017). This was related to the extraction efficiency of these components. Conversely, ginger and Indian gooseberry (*Phyllanthus emblica* officinalis) extracts showed the same range of total antioxidant and scavenging capacity but different functional effects (Kulsum et al. 2018). Ginger has shown less selective antiproliferative effects than Indian gooseberry extract.

The findings of our study also showed that the ginger extract GE2 had higher anti-radical activity than GE1 at high concentrations (500 and 1000 μgmL^{-1}). The UHPLC analysis showed that GE2 contains more 6-shogaol and less 6-gingerol than GE1, which contains less 6-shogoal but more 6-gingerol than GE2. These results are in accordance with those of a previous study that showed that 6-shogaol scavenges the DPPH radical more effectively than 6-gingerol (Dugasani et al. 2010; Guo et al. 2014).

Our results also showed that the free radical scavenging activities of the ginger extracts GE1 and GE2 increased with increasing concentration. This difference may be related to the composition of the extracts, which showed different percentages of 6-gingerol and 6-shogoal. Furthermore, 6-gingerol and 6-shogoal have different chemical structures, which may also contribute to the different antioxidant properties. A previous study reported that the conjugation of the α,β-unsaturated ketone skeleton in the chemical structure of 6-shogaol resulted in 6-shogaol having higher efficacy and potency than 6-gingerol in terms of its anti-inflammatory, anticancer, anti-emetic, and antioxidant activities (Kou et al. 2018).

The extraction method used for ginger also affects its antioxidant properties (Ho et al. 2018). Rigane et al. (2018) demonstrated that the methanol extraction method produced ginger extract with higher antioxidant activity compared to other extraction methods. This observation was supported by a previous study performed by Tohma et al. (2017). It has been shown that the ethanolic extract of ginger displayed higher Fe^{2+} reducing power than the aqueous extract of ginger. The higher antioxidant activity in the alcohol extract may be due to the high polarity of alcohol, which allows the accumulation of antioxidant compounds in the extract.

The condition of the ginger also affects the antioxidant activity. Nadeem et al. (2012) reported that dried ginger samples result in a higher percentage of inhibition in the FRAP assay than fresh ginger, which showed that the dried ginger had high antioxidant activity. The antioxidant compounds in dried ginger might undergo an oxidation process that contributes to the high antioxidant activity. This was reinforced by a previous finding that demonstrated that fresh ginger had the lowest antioxidant activity compared to dried ginger, carbonized ginger, and stir-fried ginger (Li et al. 2016). It has been suggested that the antioxidant activity of ginger is correlated with the total phenolic content. A previous study showed that the antioxidant activity was linearly proportional to the total phenolic content of ginger (Ghasemzadeh et al. 2016).

CELL VIABILITY ASSAY

Figures 3 and 4 show the percentages of viable myoblast cells (young, pre-senescent and senescent myoblasts) after incubation with GE1 and GE2, respectively, at various concentrations (0 - 1000 μgmL^{-1}) for 24 h. The percentage of viable young myoblasts was significantly increased after GE1 treatment at concentrations of 10 - 1000 μgmL^{-1} ($p < 0.05$), as displayed in Figure 3(A). For pre-senescent myoblasts, there was no significant difference observed in cell viability after treatment with GE1 (Figure 3(B)). Moreover, the percentage of viable senescent myoblasts treated with GE1 was significantly increased at concentrations of 200 - 1000 μgmL^{-1} (Figure 3(C)). For GE2 treatment, there was a significant increase ($p < 0.05$) in the percentage of viable young myoblasts treated with GE2 at concentrations of 100 - 1000 μgmL^{-1}, as shown in Figure 4(A). Figure 4(B) shows that there was no significant difference in presenescent myoblasts treated with GE2. For senescent myoblasts treated with

100 - 1000 µgmL^{-1} GE2, a significant difference ($p < 0.05$) was observed, as shown in Figure 4(C).

Cell viability was assessed by an MTS calorimetric assay to determine the effect of various concentrations of GE1 and GE2, which contain different percentages of 6-gingerol and 6-shogaol, on the percentage of viable myoblast cells in culture. The principle of this assay was based on the conversion of tetrazolium salt by succinate dehydrogenase into insoluble purple formazan, which reflected mitochondrial activity (Ahmad et al. 2006). The viability of cells is indicated by the amount of formazan produced. Myoblasts of three different ages (young, presenescent and senescent) were used in this study. The effect of GE1 and GE2 on cell proliferation was observed. Our results showed that the percentage of viable cells was increased after both GE1 and GE2 treatments. Both young and senescent myoblasts showed a similar pattern of increase in cell viability with increasing concentrations of the ginger extracts. Our findings also showed that the cell viability gradually increased in a dose-dependent manner, and no toxicity was observed at the concentrations of ginger extract used in this study. The findings of this study are summarised in Figure 5.

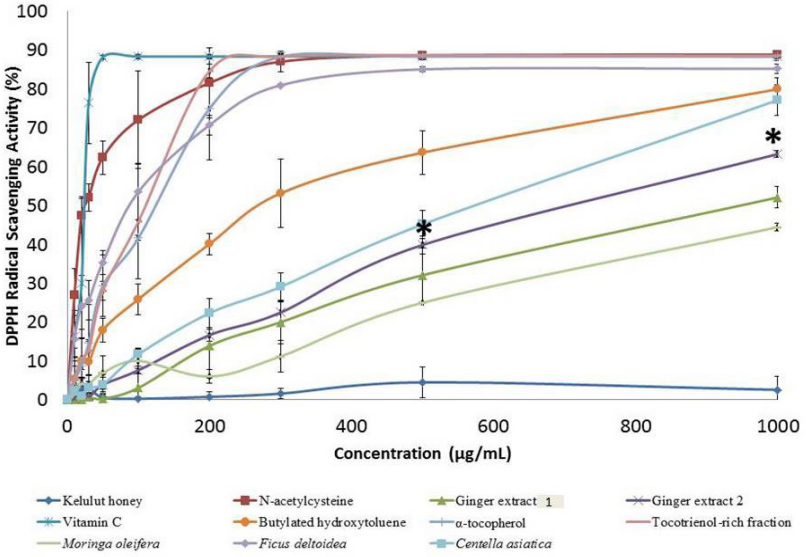


FIGURE 1. DPPH scavenging activities of ginger extract 1 (GE1), ginger extract 2 (GE2), the natural product extracts and the controls. *p < 0.05 compared to ginger extract 1

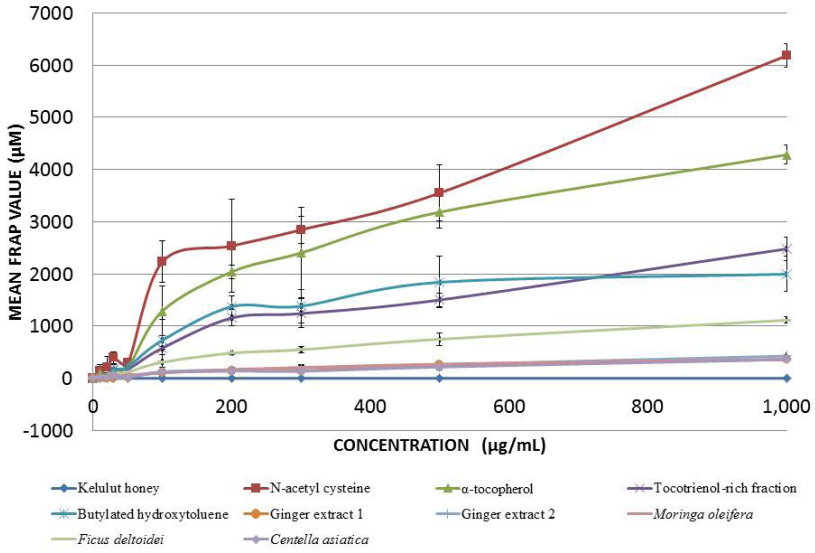
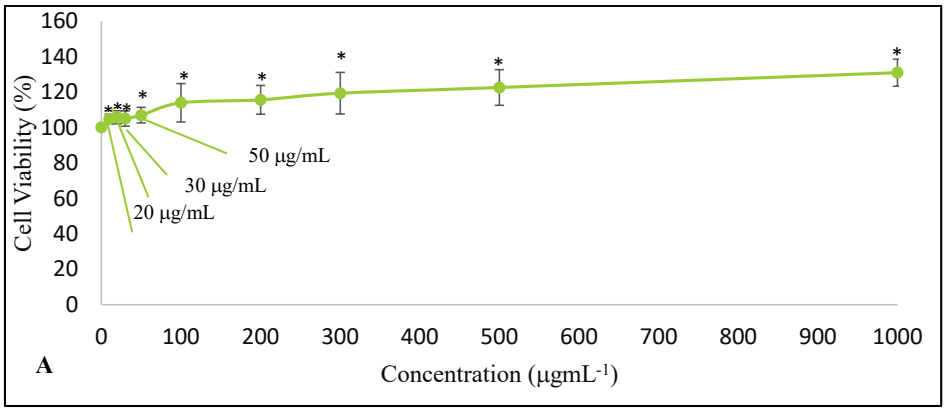
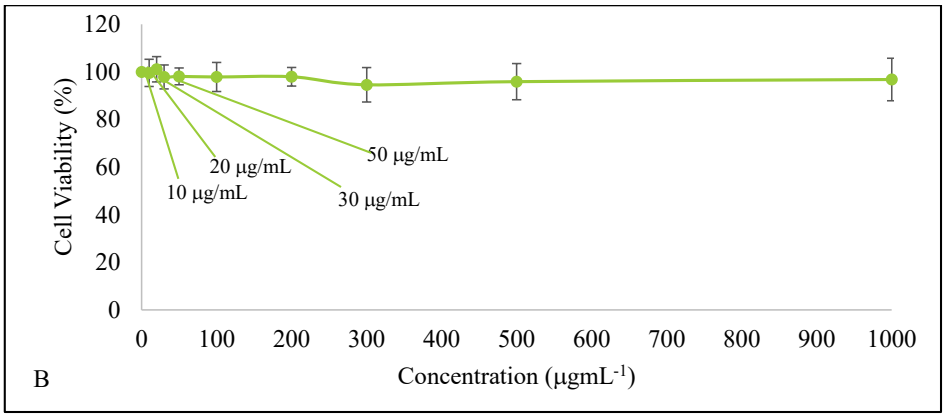


FIGURE 2. FRAP activities of ginger extract 1 (GE1), ginger extract 2 (GE2), the natural product extracts and controls





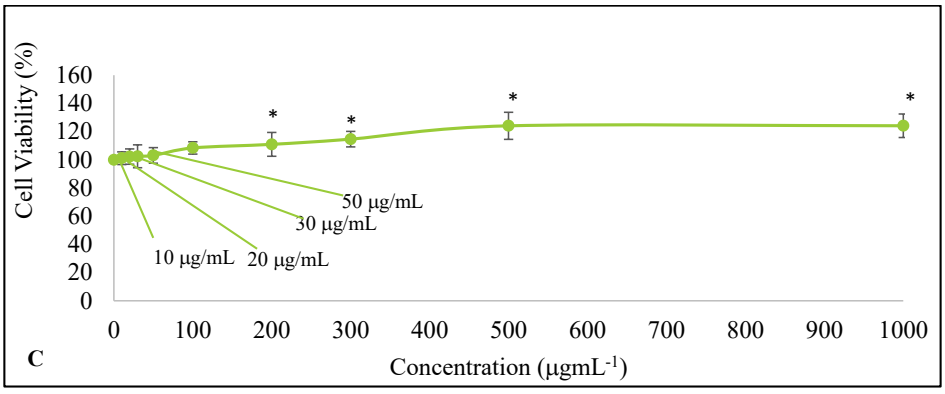
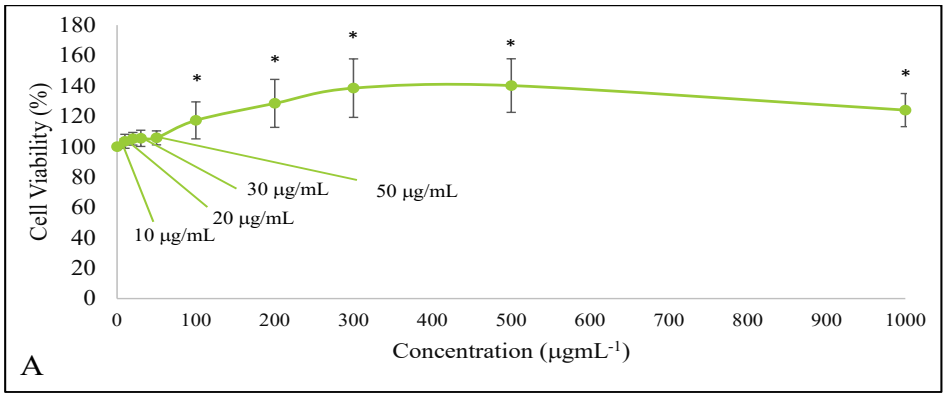
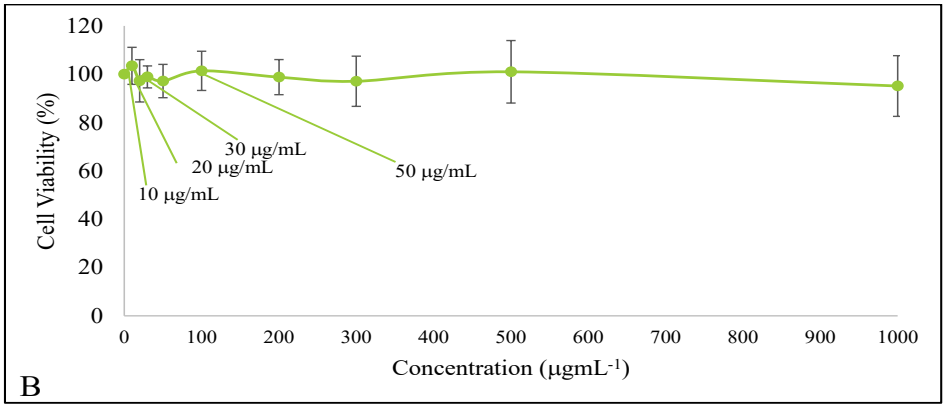


FIGURE 3. Percentage of viable young (A), presenescent (B) and senescent (C) myoblasts after incubation with ginger extract 1 (GE1). *P < 0.05 compared to 0 µgmL^{-1}. Data are expressed as the means ± SD (N=3)





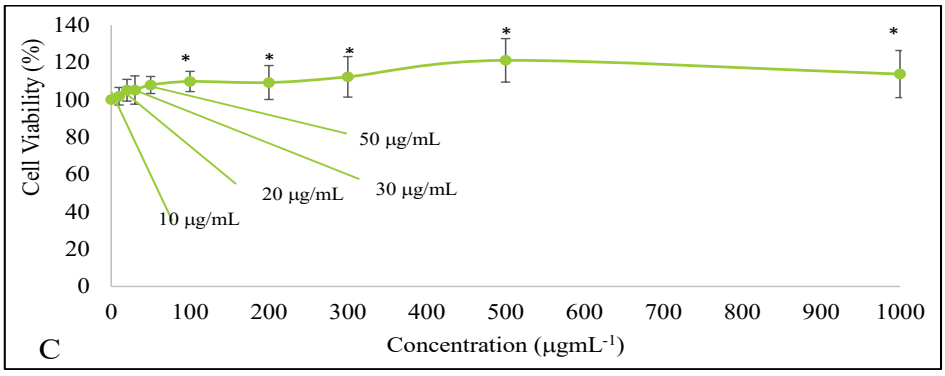


FIGURE 4. Percentage of viable young (A), presenescent (B) and senescent (C) myoblasts after incubation with ginger extract 2 (GE2). *$p < 0.05$ compared to 0 μgmL^{-1}. Data are expressed as the means ± SD (N=3)

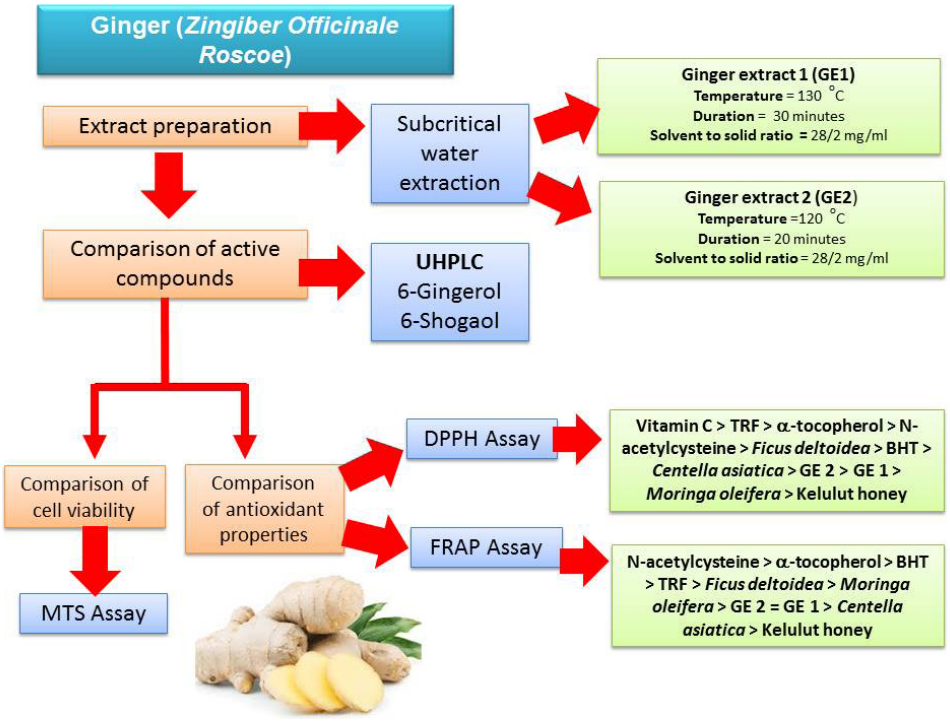


FIGURE 5. Summary of the findings

TABLE 1. Concentrations of 6-gingerol and 6-shogaol in ginger extract 1 (GE1) and ginger extract 2 (GE2) for the intra-day and inter-day assays

Compound	6-Gingerol		6-Shogoal	
	Intra-day	Inter-day	Intra-day	Inter-day
Concentration in GE1 (μgmL^{-1})	289.531 ± 2.887	266.033 ± 25.433	15.466 ± 0.271	16.333 ± 4.124
Concentration in GE2 (μgmL^{-1})	181.257 ± 1.080	167.044 ± 18.211	63.425 ± 0.239	55.943 ± 9.620

CONCLUSION

Both types of Malaysian *Zingiber officinale* Roscoe extracts possessed comparable antioxidant properties and demonstrated the ability to reduce reactive oxygen species, indicating their potential for ameliorating oxidative stress-related diseases in addition to their lack of cytotoxicity toward cells and promotion of cell viability.

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