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Research article

Growth, bioactive compound accumulation and antioxidant activity in rhizomes and storage roots of *Boesenbergia rotunda* (L.) Mansf.

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Abstract

Importance of the work: *Boesenbergia rotunda* (L.) Mansf. is one of the most important plants in traditional Thai medicine. Therefore, the choice of harvesting time is crucial to meet the needs of the pharmaceutical sector.

Objectives: To investigate the growth, bioactive compound accumulation and antioxidant activity in the rhizomes and storage roots of *B. rotunda*.

Materials & Methods: The development of both aboveground and underground parts was tracked 2–11 mth after planting (MAP) and the accumulation of bioactive compounds in the rhizomes and storage roots was tracked 6–11 MAP.

Results: Shoots and leaves developed rapidly until 7 MAP, at which point senescence began; these organs had completely dried-out by 8 MAP. Rhizomes and storage roots entered maturity at 7–8 MAP and then entered senescence. The contents of pinostrobin (rhizomes: 78.77 ± 10.07–94.13 ± 4.33 mg/g dried extract (DE); storage roots: 114.02 ± 3.00–126.64 ± 8.69 mg/g DE) and of total flavonoids (rhizomes: 204.35 ± 4.46–239.54 ± 28.07; storage roots: 181.88 ± 9.70–256.59±7.35 mg quercetin equivalents/g DE) did not vary significantly during 6–8 MAP, but decreased during 9–11 MAP. The total phenolic accumulation was stable in the rhizomes during 6–11 MAP, whereas that in storage roots decreased from 9 MAP. The strongest antioxidant activity was observed in the storage roots with a 50% antioxidant effect value of 18.27 ± 0.70 µg/mL compared to the rhizomes of 12.55 ± 1.02 µg/mL, when harvested at 8 MAP.

Main finding: For medicinal purposes, the rhizomes and storage roots of *B. rotunda* should be harvested at 7–8 MAP.

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Introduction

Boesenbergia rotunda (L.) Mansf. or fingerroot is a medicinal plant of the family Zingiberaceae that is grown in tropical areas of China, South Asia and Southeast Asia (Chahyadi et al., 2014). Its rhizomes and storage roots are used as a spice and vegetable and as food ingredients in Thailand, Malaysia, Indonesia, India and China (Eng-Chong et al., 2012; Chahyadi et al., 2014). It has also found traditional uses in the treatment of rheumatism, muscle pain, febrifuge, gout, gastrointestinal disorders, flatulence, carminative, stomachache, dyspepsia and osteoporosis (Eng-Chong et al., 2012; Gu et al., 2017; Saah et al., 2021). *B. rotunda* has been shown to yield bioactive compounds including flavonoids, chalcone derivatives, esters, terpenes and terpenoids (Eng-Chong et al., 2012). In addition, pinostrobin or 5-hydroxy-7-methoxyflavanone is the major flavonoid found in the rhizomes and storage roots of *B. rotunda* (Bhamarapravati et al., 2006; Tan et al., 2015; Patel et al., 2016; Yusuf et al., 2018). These have demonstrated pharmaceutical applications as an antimicrobial (Jitvaropas et al., 2012), antioxidant (Jitvaropas et al., 2012; Chahyadi et al., 2014), antiviral (Kanjanasirirat et al., 2020), anti-allergic (Madaka and Tewtrakul, 2011) and aphrodisiac (Ongwisespaiboon and Jiraungkoorskul, 2017). Kanjanasirirat et al. (2020) reported potent anti-SARS-CoV-2 activity by rhizome extracts from *B. rotunda*, including the suppression of SARS-CoV-2 infectivity in Vero E6 cells. The ethanolic extract from *B. rotunda* rhizome has demonstrated uses as an antioxidant and antimicrobial and for accelerating wound-healing (Jitvaropas et al., 2012). Ruttanapattanakul et al. (2021) reported that *B. rotunda* extract enhanced cell proliferation and wound healing by stimulating the MAPK and PI3K/Akt signal transduction pathways. The ethanolic extract has also been shown to promote osteoblastic cell viability and differentiation, as well as induced mineralization, in MC3T3-E1 cells, making it a potential treatment for osteoporosis (Saah et al., 2021).

B. rotunda is a small perennial plant that is propagated by rhizomes, for which it is traditionally cultivated. Nutrients and bioactive compounds are stored in the underground part and are affected by many factors, especially the age of the plant (Rawat et al., 2018). The rhizomes of species within the family Zingiberaceae show great variation in bioactive compound accumulation at different developmental stages. Policegoudra et al. (2007) documented an increase in both antioxidant activity and difurocumenonol and phenolic accumulation in the rhizomes of *Curcuma amada* throughout the developmental stages, reaching maxima 180 d after planting (DAP); these loadings declined as the plants reached senescence. The highest yields of total phenolics and flavonoids from rhizomes of *Kaempferia parviflora* were obtained from plants aged 8 mth and yields then decreased at 10 mth and 12 mth (Rahman et al., 2018). The total phenolic and flavonoid contents of *Zingiber zerumbet* rhizome increased significantly during maturation from age 3–9 mth (Ghasemzadeh et al., 2016). These results suggest that the harvesting time is a key factor in bioactive compound accumulation and therefore in the yield of medicinal materials. Therefore, to meet the needs of the pharmaceutical sector, the choice of harvesting time is crucial. To date, little has been reported on the accumulation of bioactive compounds and antioxidants in the rhizomes and storage roots of *B. rotunda*, which is an essential prerequisite in determining the optimal time to harvest. The goal of the current study was to investigate plant growth and the choice of harvesting times on yields of pinostrobin, total phenolics and flavonoids and to test the antioxidant activity of extracts from the rhizomes and storage roots of *B. rotunda*.

Materials and Methods

Plant cultivation

Seed rhizomes of the *B. rotunda* cultivar Raak-kluay were collected in Lopburi province, Thailand. Initial planting was in April 2019, then at 2 mth after planting (MAP), seedlings were transplanted into 25 cm × 50 cm planting bags with a commercial substrate comprising loamy sand with a pH of 4.84, electrical conductivity of 0.94 dS/m and concentrations of 0.31 mg/kg N, 110.95 mg/kg P and 34.00 mg/kg K. As base fertilizer 200 g/plant of manual fertilizer and 10 g/plant of 16-16-16 (N-P-K) were applied. Plants were further fertilized with 13−13−21 for 10 g/plant at 4 MAP and 6 MAP. Soil moisture was maintained using daily irrigation, which was discontinued when the aboveground plant withered and collapsed in December 2019. In total, 200 plants were grown in a greenhouse under 50% shading located at Thammasat University, Rangsit campus, Pathum Thani, Thailand.

Plant growth measurement

The experiment used a completely randomized design with four replicates. There were 7 treatments for harvesting times

(2–8 MAP) for aboveground growth and 10 for harvesting times from 2–11 MAP for underground growth. In total, eight plants were harvested at monthly intervals between 2 MAP (June 2019) and 11 MAP (March 2020). Data were collected on both the underground and aboveground parts. Shoots longer than 5 cm were counted and plant height was measured. The leaves were counted when the petiole became visible. The fresh weight (FW) of the whole aboveground part (shoot) was measured, followed by the dry weight (DW) after drying in a hot-air oven at 50°C for 72 hr.

The underground part was carefully washed and the rhizomes and storage roots were separated. Counts were made of rhizomes with a diameter greater than 1.0 cm and storage roots with a diameter greater than 0.5 cm. The FW and DW values of rhizomes and storage roots were recorded. They were dried in a hot-air oven at 50°C for 72 hr.

Preparation for alcoholic extraction

Six harvesting times (6–11 MAP) were conducted to determine the changes in bioactive compounds and antioxidant activity in the rhizomes and storage roots. Dried samples were used for alcoholic extraction. The procedure was modified from that of Rithichai et al. (2021). Each ground sample (5 g) was extracted three times with 95% ethanol at a ratio of 1:3 volume per volume for 72 hr and passed through filter paper. The combined extracts were evaporated at 50°C for 72 hr and the dried extract samples were kept at -20°C for further use.

Determination of total phenolic and flavonoid contents

The total phenolic content was analyzed using the Folin-Ciocalteu colorimetric method described by Jirakiattikul et al. (2016). The total flavonoid content was determined using a modified method of Zhu et al. (2010). A microplate reader (Power Wave XS; Biotek) was used to determine the total flavonoid and phenolic contents at 510 and 765 nm absorbances, respectively. The total phenolic content was expressed as milligrams gallic acid equivalents per gram of dry extract (mg GAE/g DE). Total flavonoid was expressed as milligrams quercetin equivalents per gram of dry extract (mg QE/g DE).

Determination of pinostrobin content

The pinostrobin content was analyzed following Yusuf et al. (2018) with modifications. Dried extract was dissolved in ethanol of high-performance liquid chromatography (HPLC) grade at a concentration of 1 mg/mL, sonicated for 1 min and then passed through a 0.22 µm membrane filter. An ultra-HPLC model (Nexera LC-30A; Shimadzu), was used to analyze the pinostrobin using a Nova-Pak C18 column (150 mm × 3.9 mm and 4 µm diameter) with a guard column and a diode array detector at 288 nm (Fig. 1). Each sample (20 µL) was injected into the system with a flow rate of 1.5 mL/min. The gradient mode was operated using a mobile phase of 0.1% phosphoric acid (A) and acetonitrile (B) as: 20% B for 0.5 min, 35% B for 4.5 min, 60% B for 5.0 min and 100% B for 8 min. The pinostrobin content was expressed as milligrams per gram of dry extract (mg/g DE).

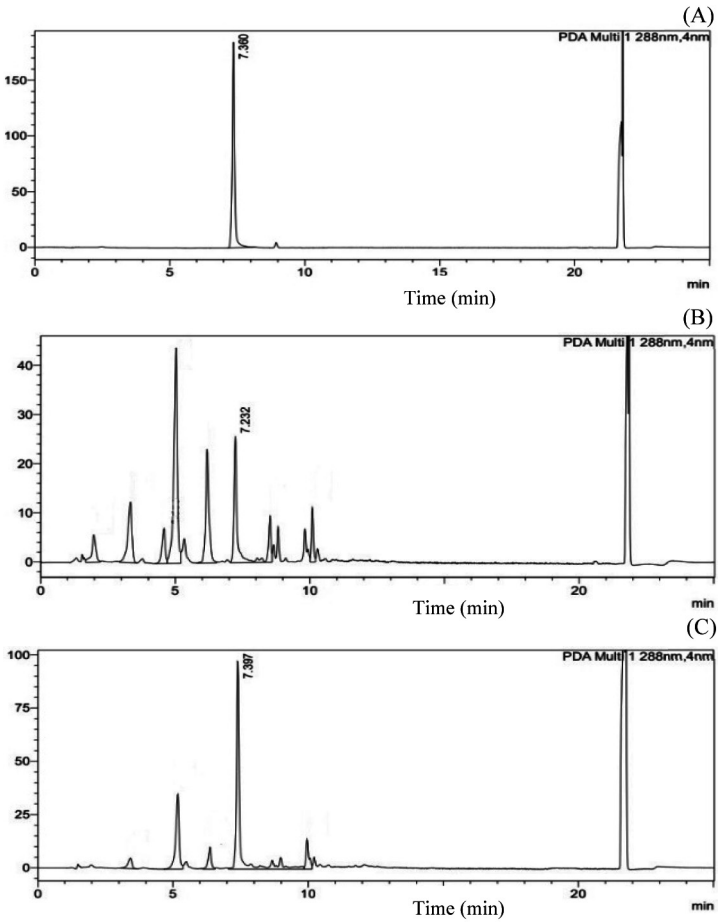

Fig. 1 High-performance liquid chromatograms of pinostrobin: (A) standard; (B) rhizome at 7 months after planting (MAP); (C) storage root at 7 MAP

Antioxidant activity

The antioxidant activity was analyzed using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity method adapted from Jirakiattikul et al. (2016). Absorbance was measured using a microplate reader at 520 nm. The values of the concentration of sample required to scavenge 50% of DPPH free radicals (EC_{50}), were calculated using a regression equation. Butylated hydroxytoluene (BHT) was used as a positive control.

Statistical analysis

The data were subjected to analysis of variance. Then means were compared using Tukey's honestly significant difference test. The tests were considered significant at $p < 0.05$. Correlation between bioactive compounds and antioxidant activity in the rhizomes and storage roots of *B. rotunda* were performed using Pearson's correlation test.

Results

Plant growth

The shoot number, leaf number, shoot FW and shoot DW increased steadily during 2–4 MAP, then rapidly to 7 MAP (Figs. 2A−2D). Plant height increased rapidly during 2–5 MAP, then slowly to 7 MAP (Fig. 2E). At 7 MAP, maxima were reached in shoot numbers (23.33 ± 2.02), leaf numbers (82.17 ± 5.01), shoot FW (1.68 ± 0.08 kg/plant), shoot DW (209.58 ± 18.55 g/plant) and plant height (39.57 ± 0.10 cm). Then, the shoots and leaves turned brown and withered, becoming completely dry by 8 MAP.

Rhizome initiation began at 3 MAP; concurrently, storage roots formed. The growth of underground parts was rapid during 4–7 MAP, where maxima in rhizome (23.33 ± 2.02) and storage root (104.33 ± 2.52) numbers, rhizome (130.25 ± 22.44 g/plant) and storage root (554.12 ± 41.63 g/plant) FWs, and rhizome (27.38 ± 1.81 g/plant) and storage root (97.09 ± 6.96 g/plant) DWs were recorded. The numbers, FWs and DWs of both rhizomes and storage roots decreased during 9–11 MAP (Fig. 3).

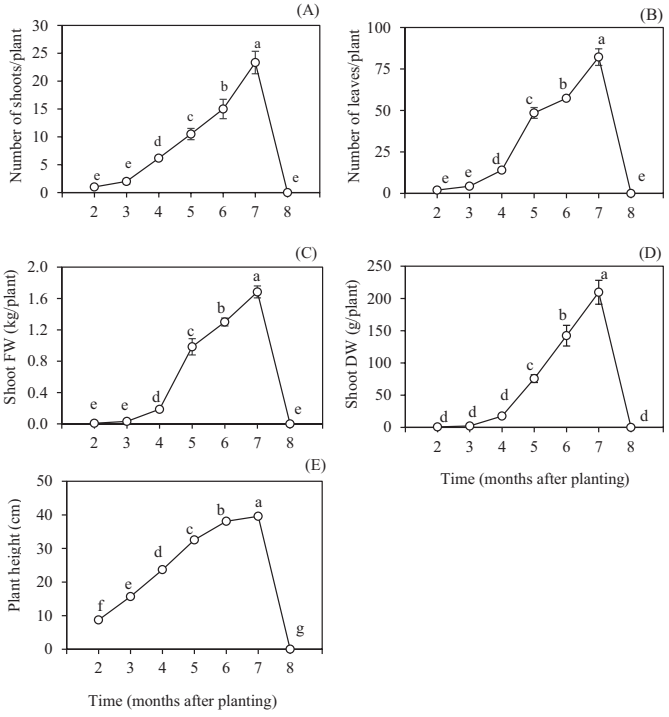

Fig. 2 Growth of aboveground part during 2–8 months after planting of *Boesenbergia rotunda*: (A) number of shoots; (B) number of leaves; (C) shoot fresh weight (FW); (D) shoot dry weight (DW); (E) plant height, where data are means of four replicate samples and error bars indicate ± SD. Different lowercase letters indicating significant ($p < 0.05$) difference

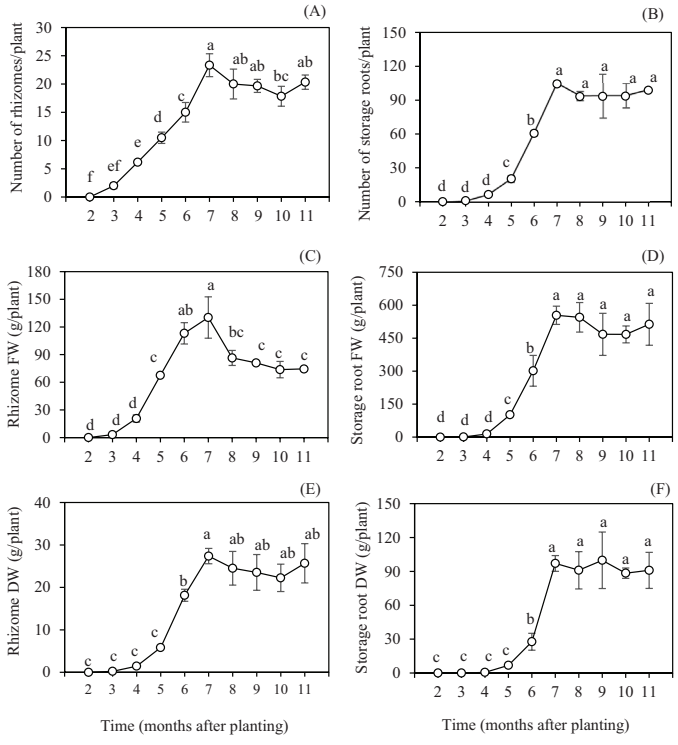

Fig. 3 Changes of underground part during 2–11 months after planting of *Boesenbergia rotunda*: (A) number of rhizomes; (B) number of storage roots; (C) rhizome fresh weight (FW); (D) storage root FW; (E) rhizome dry weight (DW); (F) storage root DW, where data are means of four replicate samples and error bars indicate ± SD. Different lowercase letters indicate significant ($p < 0.05$) difference

Bioactive compounds and antioxidant activity in rhizomes and storage roots

The extraction yields from the rhizomes and storage roots harvested during 6–11 MAP were not significantly different. The rhizome extract yield ranged from 12.72 ± 1.70% to 14.09 ± 0.32% and the storage root extract from 12.91 ± 0.26% to 14.09 ± 0.41% (Fig. 4).

During 6–11 MAP, there were no significant differences in the total phenolic contents of rhizomes, with values in the range 141.58 ± 1.22–200.45 ± 48.76 mg GAE/g DE. However, there were significant differences in the total phenolic contents of storage roots.

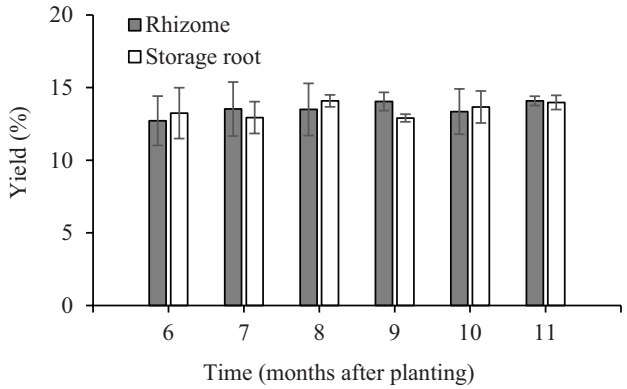

Fig. 4 Changes in extraction yield in rhizomes and storage roots of *Boesenbergia rotunda* during 6–11 months after planting, where data are means of four replicate samples and error bars indicate ± SD

The highest value of 168.61 ± 6.88 mg GAE/g DE was recorded at 6 MAP and the lowest (128.91 ± 5.64 mg GAE/g DE) at 10 MAP, with the period of decrease during 9–11 MAP (Fig. 5A).

Rhizomes had a high total flavonoid content at 6–8 MAP, in the range 204.35 ± 4.46–239.54 ± 28.07 mg QE/g DE. Total flavonoids then decreased during 9–11 MAP, falling to 157.28 ± 12.80 mg QE/g DE. The storage roots had a total flavonoid content of 181.88 ± 9.70 mg QE/g DE at 6 MAP. This increased significantly to 256.59 ± 7.35 mg QE/g DE at 7 MAP and then declined to 158.84 ± 13.34 mg QE/g DE by 11 MAP (Fig. 5B).

Pinostrobin accumulation in both the rhizomes and storage roots took place mainly during 6–8 MAP, in the ranges 78.77 ± 10.07–94.13 ± 4.33 mg/g DE and 114.02 ± 3.00–126.64 ± 8.69 mg/g DE, respectively. This declined from 9 MAP, reaching its lowest concentration of 60.95 ± 6.77 mg/g DE in rhizomes and 54.82 ± 3.86 mg/g DE in storage roots at 11 MAP (Fig. 5C).

The strongest antioxidant activity was in the rhizomes harvested at 8 MAP, with an EC_{50} of 12.55 ± 1.02 µg/mL. The rhizomes harvested during 9–10 MAP had EC_{50} values of 17.50 ± 0.78 and 17.23 ± 0.33 µg/mL, respectively. This was lower than that of BHT (EC_{50} = 17.60±1.29 µg/mL). Conversely, EC_{50} values for rhizomes harvested at 6, 7 and 11 MAP were high. Extracts from storage roots had EC_{50} values ranging from 18.27±0.70 to 43.77±2.78 µg/mL, higher than that of the positive control (BHT, EC_{50} = 13.56 ± 1.02 µg/mL) at all harvesting times. However, the greatest antioxidant activity (EC_{50} of 18.27 ± 0.70 g/ mL) was in storage roots harvested at 8 MAP (Fig. 5D).

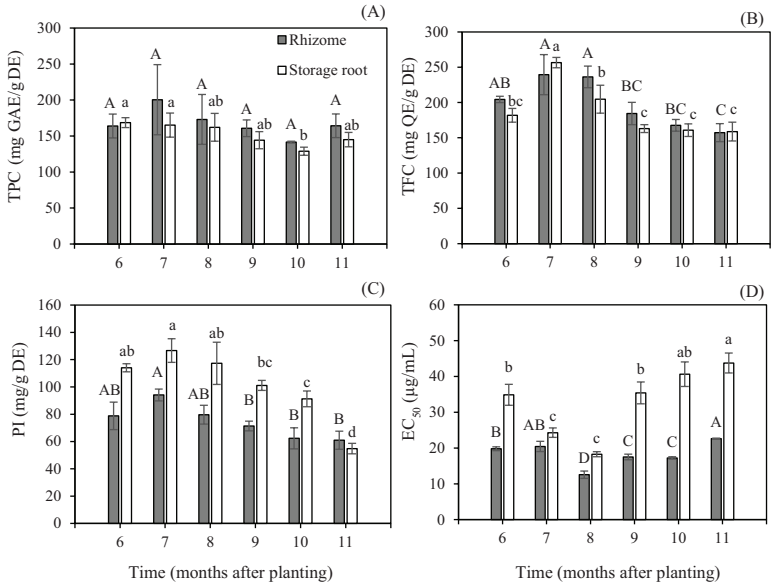

Fig. 5 Changes in rhizomes and storage roots of *Boesenbergia rotunda* during 6–11 MAP (A) total phenolic content (TPC); (B) total flavonoid content (TFC); (C) pinostrobin (PI); (D) antioxidant activity (EC_{50} = 50% antioxidant effect), where GAE is gallic acid equivalents, QE is quercetin equivalents, DE is dried extract, data are means of four replicate samples except for three replicates for pinostrobin and error bars indicate ± SD. Different lowercase or uppercase letters indicate significant ($p < 0.05$) difference among rhizomes or storage roots, respectively.

Correlation between bioactive compounds and antioxidant activity

The pinostrobin and total flavonoid contents in the storage roots had strong (r = −0.741) and moderate (r = −0.453) negative correlations, respectively, with antioxidant activity, whereas, the bioactive compounds in the rhizomes did not show any significant correlation with antioxidant activity (Table 1). This indicated that the higher contents of total flavonoid and pinostrobin in the storage roots resulted in stronger antioxidant activity. On the other hand, the antioxidant activity in the rhizomes may have been influenced by other bioactive compounds.

Table 1 Pearson's correlation coefficient of bioactive compounds and antioxidant activities in rhizomes (above diagonal) and storage roots (below diagonal) at different harvesting times

Parameter	Pearson's correlation coefficient			
	TPC	TFC	PI	AA
TPC	1	0.426^{ns}	0.250^{ns}	0.169^{ns}
TFC	0.564^{**}	1	0.657^{**}	-0.387^{ns}
PI	0.421^{ns}	0.563^{**}	1	-0.120^{ns}
AA	-0.426^{ns}	-0.453^{*}	-0.741^{**}	1

TPC = total phenolic content; TFC = total flavonoid content; PI = pinostrobin; AA = antioxidant activity

*,** = correlations are significant ($p < 0.05$) and highly significant ($p < 0.01$), respectively; ^{ns} = non-significant ($p \geq 0.05$)

Discussion

Perennial rhizomatous plants can grow for many years, with alternating periods of aboveground growth and of storage in the underground part, as well as periods of dormancy (Lebot, 2009). The rhizomes and storage roots of *B. rotunda* are utilized as food ingredients and traditional medicines (Eng-Chong et al., 2012; Jitvaropas et al., 2012; Chahyadi et al., 2014). When field-grown, the vegetative rhizomes are mainly used and cultivated during the rainy season, so in the current study, the rhizomes were planted in April and sprouting occurred 40 DAP. The aboveground part grew rapidly during 3–7 MAP. Rhizome and storage root initiation began at 3 MAP and growth was rapid from 4 to 7 MAP. In this phase, nutrients were translocated to the underground part (Lebot, 2009). The enhanced growth of stems and leaves supported rapid development of rhizomes and storage roots. The aboveground part achieved maximum growth at 7 MAP, then senescence took hold and stems and leaves turned brown, withered and were completely dry by 8 MAP. Senescence was initiated by dry conditions and low temperatures in the period November–March, at the end of which, the experiment finished. The ending of shoot vegetative growth also ended photosynthate translocation to the underground part. During 9–11 MAP, the underground part entered dormancy and the dry matter in rhizomes and storage roots decreased. Maturation of rhizomatous crops is usually determined by the leaves turning yellow, fading, and withering (Sivaraman, 2007). Based on the current results, the underground part of *B. rotunda* achieved maturation during 7–8 MAP, followed by dormancy during 9–11 MAP. The growth cycle of *B. rotunda* observed in this study was consistent with that of other rhizomatous crops, including *Curcuma amada* (Policegoudra et al., 2007) and *C. longa* (Sivaraman, 2007).

The current study was the first to track changes in the accumulation of the total phenolic, total flavonoid and pinostrobin contents, including antioxidant activities in the rhizomes and storage roots of *B. rotunda* at different points from immaturity to dormancy. The total phenolics and flavonoids represent large groups of bioactive compounds. These constituents are biosynthesized from phenylalanine generated through shikimate and phenylpropanoid pathways (Kabera et al., 2014). Ghasemzadeh et al. (2014) reported that the enzyme chalcone synthase was a key enzyme for flavonoid metabolism in plant cells. They also found an increase in chalcone synthase activity caused increments in the polyphenolic compounds in buds and leaves of *Clinacanthus nutans* (Ghasemzadeh et al., 2014) as well as those in rhizomes and leaves of *Zingiber zerumbet* (Ghasemzadeh et al., 2016). Phenolics and flavonoids are known to possess health benefits, such as antioxidants, anti-inflammatories and anti-carcinogenic, and may also offer protection against oxidative stress and some diseases (Kabera et al., 2014). Rhizomatous plants generally accumulate phenolics and flavonoids at low concentrations in the immature stage (Policegoudra et al., 2007; Ghasemzadeh et al., 2016). However, the current study showed that the total phenolic and flavonoid contents of the immature rhizomes and storage roots of *B. rotunda* at 6 MAP were not significantly different from those of more mature plants harvested at 7 MAP and 8 MAP. The rhizomes showed stable accumulation of total phenolics during 6–11 MAP, though accumulation by the storage roots decreased in the dormancy stage. The total flavonoid content of both the rhizomes and storage roots decreased in this stage. Other studies of rhizomatous plants recorded the highest levels of total phenolics and flavonoids at the maturation stage, followed by declines during senescence. For example, Policegoudra et al. (2007) recorded maxima in accumulation

of the difurocumenonol and phenolic contents at 180 DAP in *Curcuma amada* rhizomes, with decreases in the senescence stage. Rahman et al. (2018) reported that the rhizomes of *Kaempferia parviflora* contained the highest concentrations of total phenolics and flavonoids at 8 mth, decreasing by months 10 and 12. Rawat et al. (2018) reported that the total phenolic and flavonoid contents of *Roscoea procera* rhizomes reached maximum levels in the senescence and fruit maturation phases, respectively.

Pinostrobin is a flavonoid with biological uses as an anti-inflammatory, antioxidant, antimicrobial, anti-viral, anti-nociceptive, anti-cancer and anti-fungal, as well as being gastroprotective, anti-osteoporosis and a quinone reductase inducer (Bhamarapravati et al., 2006; Patel et al., 2016; Saah et al., 2021). This compound is biosynthesized through the phenylpropanoid pathway, starting with phenylalanine which is derived from the shikimate pathway (Kabera et al., 2014). Tan et al. (2015) reported that all the organs of *B. rotunda*, both aerial and non-aerial, biosynthesize pinostrobin. In the current study, the immature rhizomes and storage roots at 6 MAP had a pinostrobin content that was not significantly different from those of the more mature organs at 7 MAP and 8 MAP. In addition, the storage roots were richer in pinostrobin than the rhizomes at all harvesting times. These results were consistent with those of Saah et al. (2021), who found that the ethanolic extract from storage roots of *B. rotunda* had a higher pinostrobin level than that from the rhizomes. Conversely, Tan et al. (2015) reported the highest pinostrobin concentration in young rhizomes at the shoot base and found no significant difference in concentration between rhizomes and storage roots, whether from conventionally propagated or *in vitro*-derived plants. A lower content of pinostrobin in storage roots than that in rhizomes was also reported by Jirakiattikul et al. (2021). The reductions in the pinostrobin contents in rhizomes and storage roots during 9–11 MAP occurring during senescence of the aboveground part. Likewise, the concentration of bioactive compounds in the other plant species of the Zingiberaceae usually declined during senescence (Policegoudra et al., 2007; Rahman et al., 2018). These findings indicated that harvesting time could have an impact on the pinostrobin content in *B. rotunda*.

The antioxidant activities of extracts from the rhizomes and storage roots depended significantly on the harvesting time. For the rhizomes, the greatest antioxidant activity was observed at 8 MAP and rhizomes harvested during 8–10 MAP had stronger antioxidant activity (EC_{50} range from 12.55 ± 1.02 µg/mL to 17.50 ± 0.78 µg/mL) than the potent antioxidant BHT (EC_{50} of 17.60 ± 1.29 µg/mL). For the storage roots, antioxidant activity was again greatest at 8 MAP (EC_{50} of 18.27 ± 0.70 µg/mL), but it was not as strong as for BHT (EC_{50} of 13.56 ± 1.02 µg/mL). These results supported Thai traditional medicine practices, as rhizomes are typically collected for raw materials during the senescence stage. The evidence of DPPH radical scavenging suggests a mechanism by which rhizomes of *B. rotunda* produce antioxidant effects when harvested at 8 MAP or in the senescence of the aboveground parts. The high concentrations of bioactive compounds in the underground parts harvested during 6–8 MAP imply that these may contribute to a healthy diet or have medicinal uses. However, as the yields from the underground parts harvested at 6 MAP were too low to be of economic value, the current results indicate that the optimal time to harvest rhizomes and storage roots is during 7–8 MAP.

Rhizomes and storage roots of *B. rotunda* are useful sources of total phenolics, total flavonoids and pinostrobin and the rhizome and storage roots extracts exhibited antioxidant activities. These bioactive compounds began to accumulate in the immature underground parts but decreased during senescence. The current results suggested that the rhizomes and storage roots should be harvested during 7–8 MAP, when the aboveground part reaches maximum growth.

Conflict of Interest

The authors declare that they are no conflicts of interests.

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