Phenolic Content and Antioxidant Activity in Micro-Rhizomes of *Kaempferia parviflora*

[1]Zuraida Ab Rahman, [1]Ayu Nazreena Othman, [1]Chandradevan A/L Machap, [2]Amirah Balqis Amir Amran and [1]Nur Najwa Arifah Basiron

[1]Biotechnology & Nanotechnology Research Centre, Malaysian Agricultural Research and Development Institute, MARDI HQ, Persiaran MARDI-UPM, 43400 Serdang Selangor, Malaysia.
[2]School of Agriculture Science & Biotechnology, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200, Besut, Terengganu, Malaysia

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Abstract: The main purpose of this research is to determine the total phenolic content and antioxidant activities in micro-rhizomes of *Kaempferia parviflora*. Different parts of in vitro cultured *K. parviflora* were used in this study to equate with normal rhizomes grown naturally. The in vitro culture process was initiated using Naphthaleneacetic (NAA) and Benzyl-aminopurine (BAP) in the growth medium either on its own or combined (1 mg/L NAA, 2 mg/L NAA, and 1 mg/L NAA + 1 mg/L BAP). The in vitro cultured accumulation of total phenolic content and antioxidant activities is significantly lower as correlated to the one naturally grown *K. parviflora rhizomes.*

Keywords: *Kaempferia parviflora*, antioxidant activities, phenolic content, microrhizomes

Introduction

Kaempferia parviflora, often known as 'Thai ginseng' is a Zingiberaceae-family medicinal plant. It may be found in tropical locations including Malaysia, Sumatra, Borneo Island, and Thailand. For generations, its rhizome has been utilized as a traditional medicine to boost health. *K. parviflora* has been proven in pharmacological trials to be antimicrobial, antiallergenic[1], antidepressive[2], anti-inflammatory[3], anticancer[4,5], and antimutagenic[6]. Multiple applications of *K. parviflora* have necessitated its bulk collection as a raw material for medicines, resulting in the loss of this natural resource and increasing pressure on K. parviflora populations[7]. As a result, a sustainable cultivation strategy is required to avoid the loss of wild populations of *K. parviflora* while meeting the increased demand. *K. parviflora* rhizomes can be used to propagate the plant. However, it took about 12 months to obtain mature rhizomes[8].

The instability of bioactive compounds in plants has caused complications in the downstream process. With the establishment of efficient in vitro cell and plant regeneration techniques of *K. parviflora* the genetic improvement and mass production of valuable metabolites can be achieved. In vitro production of biologically active phytochemicals through cell and organ culture as a reliable method is essential for generating cultures within a short period throughout the year[9]. Phenolic compounds are one of the bioactive compounds found in *K. parviflora*, it is the main class of secondary metabolites in plants and is divided into phenolic acids and polyphenols. Phenolic compounds, which are natural antioxidants, are prevalent in plant foods and drinks, which play important roles in healthcare. According to several studies, phenolic compounds are the most abundant in regular human diets of all dietary antioxidants. Recently, phenolic compounds have sparked considerable attention as a result of active reports of their possible role in the prevention of several human ailments[10]. Phenolic compounds, for instance, phenolic acids and flavonoids which could be found in *K. parviflora*, could promote health benefits by reducing the risk of metabolic syndrome[11].

The goal of this study is to compare the total phenolic content and the antioxidant activity in micro-rhizomes of *K. parviflora*. Different types of *K. parviflora* micro-rhizomes were used (root, young rhizomes, mature rhizomes, and normal rhizomes) in this study with two different growth regulators, Auxin: Naphthaleneacetic acid (NAA) and Cytokinin: Benzylaminopurine (BAP). Both compounds auxin and cytokinin are responsible for cell elongation, division, differentiation, and embryonic development[12] and promote cell division and cell differentiation[13] respectively.

Materials and Methods

Initial Cultures Establishment

Kaempferia parviflora plants were cultivated inside a glasshouse located at the Malaysian Agriculture Research and Development Institute (MARDI). When rhizomes of *Kaempferia parviflora* have sprouted, buds were collected from the shoots and act as the source of explant. The shoots were cleaned under running tap water for an hour and then were washed with commercial laboratory detergent. After that, the explants were rinsed with water. The explants were submerged in 1 % (v/v) of fungicide (Benomyl 50 %, Benlate ®) for one hour and followed with rinsing with tap water. Afterward, the explants were sterilized with Clorox which is bleach and followed by few rounds of cleaning and rinsing with sterile distilled water. The leaf sheath that was present on the buds of the explants were removed using a sterile surgical blade under aseptic condition. The sterilized explants were then inoculated onto basal Murashige and Skoog's (1962) medium with 3.0 mg/L of benzyl aminopurine (BAP) for the production of plantlets in vitro. The pH of the medium was then adjusted to 5.8 before proceeding for autoclave at 121 °C under 1.05 kg/cm^2 for 15 minutes. The cultures were grown under white fluorescent light (3,000 lux) with the adjustment of photoperiod duration of 16 hours light and 8 hours darkness at 25 2 °C. The plantlets cultured in vitro were used to induce microrhizome.

Mircrorhizomes Induction

Plantlets of *Kaempferia parviflora* (Figure 1a) grown in vitro under aseptic conditions were used as explants for the induction of microrhizome. The shoots of the explants were sub-cultured in 150 ml flasks on a liquid medium consisting of MS medium supplemented with 60 % sucrose to induce microrhizome and there were three separate treatments included in this research (1 mg/L of NAA, 2 mg/L of NAA, and the combination of 1 mg/L of NAA with 1 mg/L of BAP). The cultured plants were cultured for three months but the plantlets were sub-cultured into the same liquid medium every month. After three months, the roots, young rhizomes, and matured rhizomes were collected from the treatments and proceed to determine the phenolic and antioxidant content.

Determination of Phenolic Content

The rhizomes obtained from the subculture were sliced and dried at a temperature of 40 °C – 50 °C for three days. After three days, the total phenolic content (TPC) was determined from the dried rhizomes. The TPC was determined using the Folin-Ciocalteu (FC) method. Dry rhizomes (0.5 g) were added to 0.2 ml of FC reagent (5-fold diluted with distilled water) and then were mixed continuously for 3 minutes. Sodium carbonate with a volume of 0.2 ml (10 % w/v) was added to the mixture and then the mixture was left to stand at room temperature for 30 minutes. The mixture's absorbance was read at 760 nm using Jasco V-550 UV-VIS spectrophotometer. TPC was expressed as milligram gallic acid equivalent per gram dry extract (mg GAE/mg dry extract).

Determination of Antioxidant Activity

The antioxidant activity of the mixture was determined using DPPH radical scavenging assay. 0.5 ml of microrhizome extract with 4.5 ml of DPPH were put inside a 250 ml flask alongside methanol. The mixture was then shaken and was left to stand at room temperature for 30 minutes in the dark. The antioxidant activity was determined at 517 nm against blank solvent. The antioxidant activity was recorded and compared with regarding the roots, young rhizomes, matured rhizomes, and also normal rhizomes.

Result and Discussion

Figure 1 shows the contents of total phenolics and antioxidant activity of different parts of *K. parviflora*. Some significant tissue and age-dependent differences were used. Different parts named roots (Figure 1b), young rhizomes (Figure 1c,d) and mature rhizomes (Figure 1e,f) of plantlets are cultured using different mediums (1 mg/L NAA, 2 mg/L NAA, and 1 mg/L NAA + 1 mg/L BAP). The results are then compared to a normal rhizome (Figure 1g) phenolic and antioxidant accumulation.

Based on the results shown in Figure 2, it can be seen that the accumulation of phenolic content is the highest (2088 U/g dry sample) in the concentration containing combination 1 mg/L NAA + 1 mg/L BAP. The results of

antioxidant activity are parallel with the phenolic content in Figure 1 where the combination of 1 mg/L NAA + 1 mg/L BAP recorded for the highest antioxidant activity. This suggested that the concentration of 1 mg/L NAA + 1 mg/L BAP is optimal in extracting the highest antioxidant content in *K. parviflora.* Many[14,15,16] studies have mentioned the usage of micro rhizomes of the *Kaempfria* species to identify the antioxidant level and the reason for it can be seen in the results provided in Figure 1 where rhizomes have higher phenolic and antioxidant content compared to roots of *Kaempeferia parviflora*. Similar findings can also be seen in a study[14] that stated that the antioxidant activities are the greatest in rhizomes compared to leaves of *Kaempfria* species. Furthermore, a study[15] stated that the combination of 2.69 µM NAA with 2.22 µM BAP proved that the media was superior in the production of micro rhizomes of *K. rotunda*. This further supported the combination of plant growth regulators used in this research. To further strengthen the combination of BAP and NAA as optimal plant growth regulators for *Kaempeferia parviflora,* a study[16] that used 1 mg/L NAA + 1 mg/L BAP and the percentage of micro rhizomes are the highest (15 %) compared to other concentration combinations that recorded for an average 1 % to 10 % only.

Figure 1: Micro-rhizomes initiation of Kaempferia parviflora. a) Plantlets, b) roots, c,d) Young micro-rhizomes, e,f) mature micro-rhizomes and g) normal rhizome.

The level of phenolic compounds in the extracts of roots and rhizomes of *K. parviflora* may be influenced by many aspects. Polyphenolic compounds are known to have antioxidant activity[17]and it is likely that the activity of the extracts is due to these compounds. This behavior is thought to be mostly owing to their redox properties, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxide[18]. In comparison to the mature rhizomes, the young rhizomes have a lower accumulation of total phenolic content. This is influenced by the water content, as more mature tissue (mature rhizome) has lower water content compared to the immature tissue (young rhizome)[19]. Other than that, the concentration of phenolic acids is positively correlated with tissue concentration of nitrogen and phosphorus[20]. Some[21,22] have previously said that phenolic compounds are often produced as a defense against pathogen assault or in reaction to a stressful environment and that they are not directly connected to the growth processes and development of plant tissue. This has strongly supported the fact as if comparing the samples and typical rhizomes, the samples have less total phenolic content as they are more relaxed in controlled environments. The normal rhizomes have a high accumulation of phenolic compounds because they are more stressed in nature as they need to produce phenolic compounds for their defense from pathogen attack.

The antioxidant activity of three distinct sections of K. parviflora is shown in Figure 2. K. parviflora roots, young rhizomes, and mature rhizomes are cultivated in various media (1 mg/L NAA, 2 mg/L NAA, and 1 mg/L NAA + 1 mg/L BAP). The results are then compared to rhizome accumulation in the wild. As mentioned earlier the highest antioxidant content can be seen in mature rhizomes which were recorded at 1993 U/g dry sample. Mature rhizomes are seen to have higher antioxidant activity compared to young rhizomes when compared in the same concentration used. Similar findings are also seen in a study[23] that found out that mature rhizomes have the highest percentage of free radical scavenging activity (76.42 %) compared to other parts such as leaf and stem. Other than that, the antioxidant activity of rhizomes of *K. parviflora* is superior and provides the most preferable antioxidant activity according to a previous study[24]. Therefore, this further proves that rhizomes are the most suitable parts in *K. parviflora* for the determination of antioxidant content.

Many additional elements can readily alter plant antioxidant activity, for example in sample preparation, plant parts, solvent and extraction method used[25] Many writers, on the other hand, claimed that total phenolic content has a direct correlation with antioxidant activities because phenolic compounds have a great scavenging potential to battle free radicals due to their hydroxyl groups[26]. According to several studies, intense colored plants will tend to have high antioxidant activities[27]. Based on data, normal rhizomes have the highest antioxidant activities, this might be due to the color intensity of the rhizomes is higher in comparison to the other parts. It is then supported with the study that shows that greenhouse plants exhibited more potent activities than plants from in vitro culture[28]

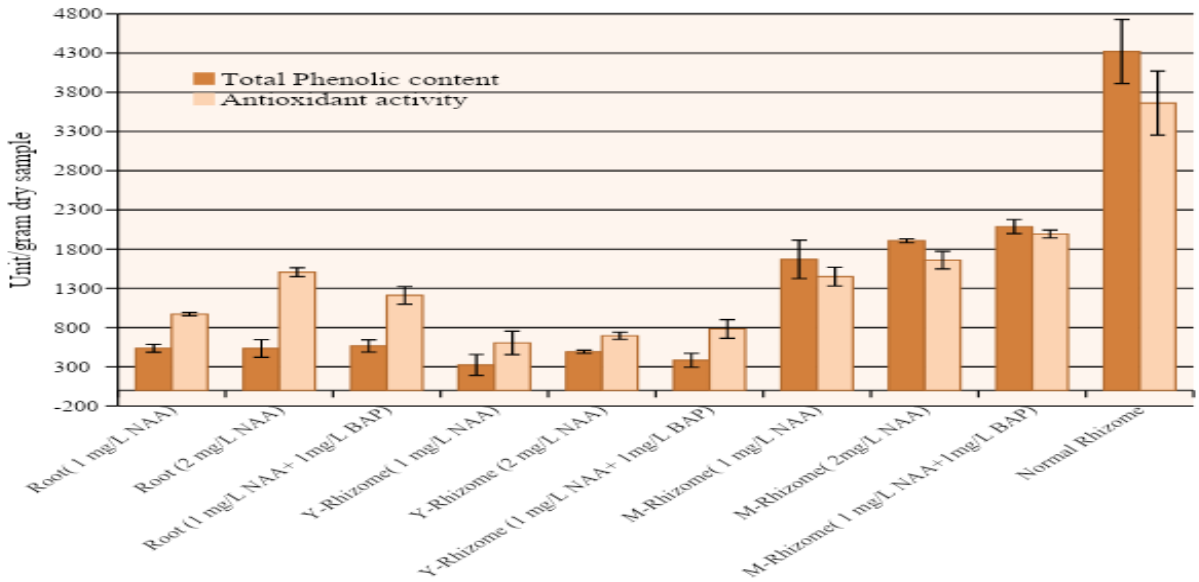

Figure 2: Total Phenolic Content and Antioxidant activity in micro-rhizomes

Conclusion

In conclusion, the study for total phenolic content and antioxidant activities in micro-rhizomes of *K. parviflora* by using various parts of rhizomes (root, young rhizomes, and normal rhizomes) and different mediums (1 mg/L NAA, 2 mg/L NAA, and 1 mg/L NAA + 1 mg/L BAP) has resulted to be related to the certain study. *K. parviflora* total phenolic content and antioxidant activities have a direct association because of phenolic compounds which have high scavenging capacity to combat free radicals. Furthermore, the combination of 1 mg/L NAA + 1 mg/L BAP is suitable in order to produce high phenolic and antioxidant activity. Other than that, this study also proved that rhizomes have better phenolic and also antioxidant content. However, in vitro cultures have a lower accumulation of phenolic compounds and antioxidant activities compared to normal rhizomes are affected by many elements such as the growth environment and physical properties.

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