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Research article

Hydroponic cultivation of black galingale (*Kaempferia parviflora* Wall. ex. Baker)

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Abstract

Black galingale (*Kaempferia parviflora* Wall ex Baker) rhizome is a popular herbal dietary supplement and traditional medicine in Asia. Mass production of high-quality rhizome is conventionally conducted in highland tropical fields. Hydroponic cultivation is a potential alternative approach that promotes environmental-friendly rhizome production. Hydroponic and potting systems were tested in two different environments: a nylon net house and an air-conditioned room. The highest black galingale yields were obtained using hydroponics with volcanic pebble at 170 g/bucket, followed by sponge (131 g/bucket), popper (98 g/bucket) and no medium control (77 g/bucket), respectively. In contrast, rhizomes grown under control conditions yielded significantly higher flavonoid contents than other treatments, with total flavone of 664 µg/g, followed by popper (442 µg/g), volcanic pebble (399 µg/g) and sponge treatments (196 µg/g), respectively.

Introduction

Kaempferia parviflora Wall ex Baker, commonly known as black galingale or 'Krachai Dam' in Thai, is a native Thai plant of the Zingiberaceae family and its rhizomes have been widely used in traditional medicine as a health-promoting herb for treating a variety of ailments with benefits including anti-mycobacterial effects (Yenjai et al., 2004), sexual dysfunction and ulcer treatment (Rujjanawate et al., 2005), anti-allergy (Tewtrakul et al., 2007), anti-flatulence and anti-dysentery (Chomchalow et al., 2003), antidepressant (Hawiset et al., 2011) and increasing blood flow to the testes (Chaturapanich et al., 2008; Tewtrakul and Subhadhirasakul, 2008). Krachai Dam exhibited aphrodisiac properties that improved the sperm density, testosterone level and sexual performance of streptozotocin-induced diabetic rats (Fungfueng et al., 2016; Lert-Amornpat et al., 2017). Gabriely et al. (2002) reported that reduced visceral fat content could prevent insulin resistance and glucose intolerance in diabetic fatty rats. In addition, there was an increase in plasma insulin levels in rats that subsequently led to reduced weight and decreased risk factors associated with type II diabetes and cardiovascular disease (Barzilai and Gupta, 1999; Resnick et al., 2000; Wing et al., 2011). Some researchers reported the presence of individual flavones in black galingale. Horikawa et al. (2012) found that 3,5,7,4′-tetramethoxyflavone and 3,5,7,3′,4′-pentamethoxyflavone enhanced adipogenesis. Jansakul et al. (2012) reported that 3,5,7,3′,4′-pentamethoxyflavone caused relaxation of human corpus cavernosum through voltage-dependent Ca^{2+} channels and other mechanisms associated with calcium mobilization. Temkitthawon et al. (2011) reported the benefit of 5,7-dimethoxyflavone as an inhibitor of the enzyme PDE5, thus enhancing sexual performance. Cultivation of

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K. parviflora in soil is the most common form of mass scale production, predominantly conducted in high-altitude areas in northern Thailand, for example in Loei, Phitsanulok and Phetchabun provinces, where one growing cycle takes approximately 9–10 mth, usually from April to December (Pojanagaroon and Kaewrak, 2009). Recently, demand for *K. parviflora* has exceeded supply, resulting in heavy exportation, either in the form of dried bulbs or extracted products. Deforestation of hills is quick and easy but is an undesirable way for farmers to expand their cultivation area; however, other important reasons for relocation result from soil-borne diseases caused by bacteria, fungi or by both pathogens (Pojanagaroon, 2010).

Hydroponics is an alternative horticultural system for all-season crop production. However, few hydroponic systems are suitable for rhizome crops as most are intended for either leaf or fruit crops with fibrous root systems or a measurable crown size and rhizome-producing crops grow differently with a horizontal growth habit which requires room to expand and produce vertical shoots and secondary roots as needed, unrestrained by physical barriers (Hayden et al., 2004).

The objective of this study was to assess the viability of *K. parviflora* cultivation utilizing a circulating hydroponic fertigation system. A conventional pot cultivation system was used for comparison with the same supporting media and granular slow-release fertilizer.

Materials and Methods

Plant materials

Rhizomes of *K. parviflora* variety Phurua-10 ('Rom-klao') aged 2 mth were obtained from a farmer at the Agricultural Extension Office, Loei, Thailand. The rhizomes had an average weight of 65.5 g and were stored at 25°C for 2 wk prior to planting.

Hydroponic system

The hydroponic system consisted of three types of supportive growth media in addition to the control (no supporting media): 1) volcanic pebble (1.0–1.5 cm diameter); 2) popper (1.0–1.5 cm diameter) (purchased at Rabeangmai shop, Bangkok, Thailand); and 3) sponge (2.5 cm^3 cubes) (purchased at Highgreen shop, Bangkok, Thailand). Twenty-four plastic buckets (45.0 cm × 55.5 cm × 35.5 cm) equipped with inlet and outlet pipes were prepared for the four treatments. Each treatment was replicated six times as a complete randomized design model. Each bucket was provided with three inverted, small, round plastic baskets (12.7 cm diameter × 15.2 cm height) for root aeration. Fig. 1A illustrates the system schematically, while Fig. 1B shows the actual planting system. All rhizomes were cut into 2.0–2.5 cm diameter pieces, cleaned using tap water and soaked in 0.6% w/v sodium hypochlorite solution (Clorox 10%) for 5 min. The rhizome pieces were then rinsed with distilled water and soaked in warm water (50°C) for 10 min to reduce nematodes, similar to the method for galingale (Trujillo, 1963). The pieces were placed in Klasmann base substrate plus medium (Klasmann-Deilmann GmbH; Geeste, Germany) and drenched with two liquid pesticides: pentachloronitrobenzene (248 g/L) + etridiazole (66 g/L) (Terrachlor super X; Crompton Corp.; Middlebury, CT, USA) and kasugamycin (2.00%) (Kasumin 2L; Arysta LifeScience; Cary, NC, USA), both at a 1:200 dilution with water. The pesticides enhanced protection against fungal and bacterial-induced wilting. The rhizomes were placed in plastic pots for 4 wk to allow shoot and root development prior to transfer into the hydroponic system. After 1 mth, all rhizomes were randomly transferred into 24 prepared plastic buckets with three rhizomes/bucket. The rhizomes were kept for 9 mth in the nutrient circulating system as shown in Table 1. The major and minor nutrients were obtained from nitrate, phosphate, chloride and sulfate salts, while ethylenediaminetetraacetic acid (EDTA) chelates (Akzo Noble BV; Sassenheim, the Netherlands) were used for trace elements, except borate and molybdate which were present as sodium salts. Two nutrient concentrations were circulated continuously, one at the starting stage and the other 3 mth later to stimulate rhizome development and accumulate active compounds. The two pesticides were added at the start of the experiment and also every time a nutrient concentration adjustment was needed.

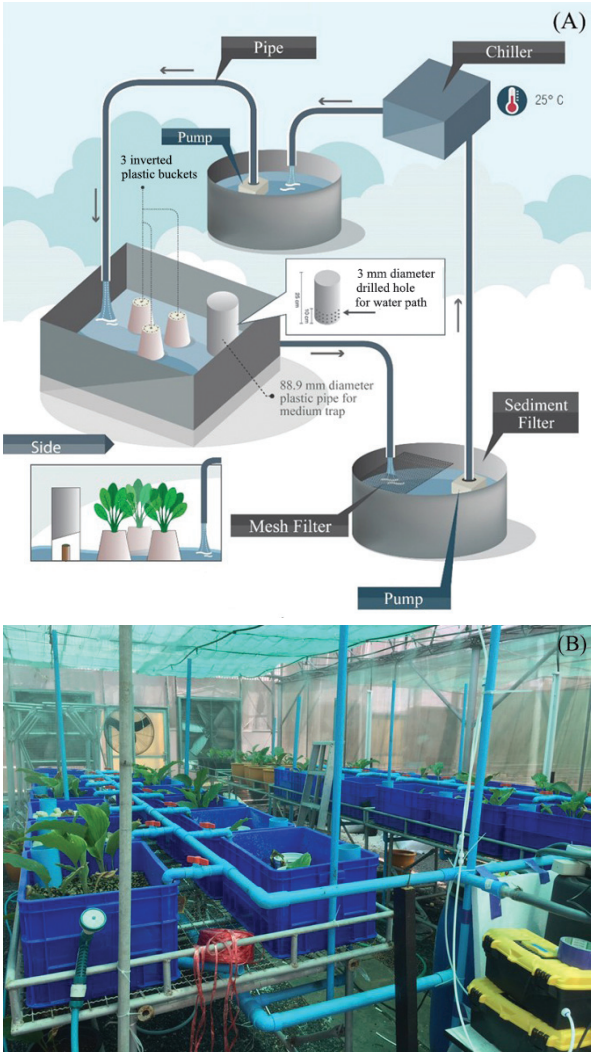


Fig. 1 Experimental hydroponic system: (A) schematic diagram; (B) actual system

Table 1 Levels of anions and cations prepared for the solutions

Element	First stage (EC* = 2.0, pH = 5.7) (Start up–3 mth; ppm)	Second stage (EC = 3.0, pH = 5.8) (3 mth or more; ppm)
Nitrate-nitrogen (N)	595.00	865.00
Phosphate (P_2O_5)	950.00	2405.00
Sulfate-sulfur (S)	410.00	535.00
Chloride (Cl)	195.00	–
Potassium oxide (K_2O)	970.00	2410.00
Calcium (Ca)	960.00	1150.00
Magnesium (Mg)	240.00	270.00
Iron (Fe)	65.00	32.50
Manganese (Mn)	4.50	32.50
Boron (B)	1.50	20.00
Copper (Cu)	0.30	2.25
Molybdenum (Mo)	0.20	1.00
Zinc (Zn)	0.30	37.50
Nickel (Ni)	0.10	0.10
Cobalt (Co)	0.10	0.30

* EC = electrical conductivity; ppm = parts per million.

The same treatments were performed at two different locations. One location was in a nylon net house with 40% black nylon shading on the top, a daytime temperature of 35°C and a night temperature of 28°C. The other location was an air-conditioned room at 25°C, equipped with light-emitting diode bulbs at 600 lux for 12 hr/d. Nutrient uptake by the plants was analyzed four times (days 1, 20, 98, 118). All analyses were performed before the nutrient concentration was adjusted. Ion chromatography (IC; ICS-2000; Dionex Corp.; Sunnyvale, CA, USA) was used to measure chloride, nitrate, phosphate and sulfate anions. Inductively coupled argon plasma (ICP; Optima 8000 ICP-OES; PerkinElmer Corp.; Waltham, MA, USA) was used to measure cations as well as boron and molybdenum. All plants were harvested after 9 mth and cleaned with tap water. The roots were cut off and air-dried to determine the average yield. The active compounds, mainly flavonoids, were obtained using 95% ethanol (Sigma-Aldrich Corp.; Burlington, MA, USA) extraction, analyzed using high performance liquid chromatography (HPLC; Waters e2695; Waters Corp.; Milford, MA, USA) and compared with seven chemical standards: 5-hydroxy-7-methoxyflavone, 5,7-dimethoxyflavone, 5,7,4′-trimethoxyflavone, 5,7,3′,4′ tetramethoxyflavone (Indofine Chemical Co Inc; Hillsborough, NJ, USA), 3,5,7-trimethoxyflavone, 3,5,7,4′-tetramethoxyflavone and 3,5,7,3′,4′-pentamethoxyflavone (Tokiwa Phytochemical Co. Ltd; Chiba, Japan). A C18 column with methanol and water at a 6:4 ratio was used as an isocratic eluent at a flow rate of 1.0 mL/min with ultraviolet detection at a wavelength of 330 nm.

Potting system

Twenty-four plastic pots (25.5 cm diameter × 30.0 cm high), each with one drainage hole, were filled with the same medium as the hydroponic system, except for the control where peat moss was used to hold the rhizome. All procedures were the same except for fertigation; in place of a nutrient circulating system, a slow-release granular fertilizer 13-13-13 (Osmocote; Scotts Corp.; Marysville, OH, USA) was applied at 5 g/plot immediately after transplanting and again 3 mth later. One hundred milliliters of the pesticide solution were drenched into every pot immediately after transplanting and repeated every month. All plants were watered every 2 d throughout the planting period. Treatments were performed in both environments (the nylon net house and the air-conditioned room).

Statistical analysis

Analysis of variance was used to analyze the data, and means were compared using least significant difference in the SAS version 9.13 software package (SAS Institute; Cary, NC, USA). Significance was set at $p < 0.05$.

Results and Discussion

Hydroponic system

Nutrient absorption by *K. parviflora* from both environments at transplanting and after 20 d are shown in Table 2. Taking into consideration that some portion of each element was adsorbed by the planting media, the starting concentrations were lower than

Table 2 Levels of anions and cations detected before and after application during first stage

Element	Nylon net house (ppm)		Air-conditioned room (ppm)	
	Start	After 20 d	Start	After 20 d
Nitrate-nitrogen (N)	201.83 ± 6.05	149.85 ± 4.49	144.67 ± 2.89	135.45 ± 2.71
Phosphate (P_2O_5)	387.63 ± 9.69	236.52 ± 7.10	394.20 ± 5.91	210.97 ± 4.22
Sulfate-sulfur (S)	160.00 ± 3.20	131.54 ± 2.63	115.55 ± 1.44	113.22 ± 1.03
Chloride (Cl)	Not detected	Not detected	Not detected	Not detected
Potassium oxide (K_2O)	< 1,000.0	< 1,000.0	Not detected	Not detected
Calcium (Ca)	383.98 ± 5.76	266.00 ± 6.65	284.51 ± 5.69	230.64 ± 3.46
Magnesium (Mg)	115.42 ± 2.31	98.86 ± 1.48	102.31 ± 2.05	72.69 ± 0.73
Iron (Fe)	31.08 ± 0.93	17.85 ± 0.22	22.68 ± 0.68	22.99 ± 0.46
Manganese (Mn)	6.16 ± 0.31	2.61 ± 0.13	6.45 ± 0.25	6.61 ± 0.09
Boron (B)	4.98 ± 0.15	4.12 ± 0.21	5.13 ± 0.07	2.23 ± 0.11
Copper (Cu)	Not detected	Not detected	Not detected	Not detected
Molybdenum (Mo)	Not detected	Not detected	Not detected	Not detected
Zinc (Zn)	13.23 ± 1.05	11.88 ± 0.17	25.23 ± 0.25	5.37 ± 0.15
Nickel (Ni)	Not detected	Not detected	Not detected	Not detected
Cobalt (Co)	Not detected	Not detected	Not detected	Not detected

ppm = parts per million.

values are presented as mean ± SE.

the prepared solution (Table 1). The major nutrients (nitrogen and phosphate) were reasonably utilized by the plants according to the IC determination. However, the ICP could not detect potassium reliably perhaps because the detection limit was set low to detect all other elements at once; therefore, potassium was out of range. The utilization of minor elements, including calcium, magnesium and sulfur, was also within reasonable proportions. The exception was the absorption of sulfur in the air-conditioned room. Clearly, concentrations of trace elements detected by ICP were much higher than those in the prepared solutions. These higher concentrations might have occurred as a result of contamination from the salts used to prepare the major and minor elements, since technical grade chemicals were used instead of laboratory grade. The absorption values of iron and manganese were higher in the nylon net house than in the air-conditioned room, while the opposite trend was observed for both boron and zinc (Table 2). The exact reason for this remains unknown.

When the nutrient solution was changed to a higher concentration at the third month, the nutrients absorbed by *K. parviflora* at both locations remained high (Table 3). Unfortunately, after the fourth month *K. parviflora* in the air-conditioned room became infected with two kinds of pathogens: *Fusarium oxysporum*, a fungal cause of wilt disease and *Ralstonia solanacearum*, a bacterial root and stem rot disease. The two pesticides applied could not prevent the outbreak of these diseases and all plants in the room died. Only plants from the nylon net house were harvested, and their yields and levels of some active compounds were compared among treatments. The yields of *K. parviflora* and the total extracted flavone contents are shown in Fig. 2. The mean yield (± SE) from the volcanic pebble treatment was the highest at 170.08 ± 32.74 g/bucket, equivalent to 6,803.2 kg/ha (approximately 40,000 buckets/ha). This was not significantly different from the sponge treatment; however, it was significantly different from the popper and control treatments. The yield from the

Table 3 Levels of anions and cations detected after the third month

Element	Nylon net house (ppm)		Air-conditioned room (ppm)	
	Starting	20 days after	Starting	20 days after
Nitrate-nitrogen (N)	205.00 ± 6.15	90.00 ± 3.24	288.45 ± 4.61	185.40 ± 3.71
Phosphate (P_2O_5)	296.38 ± 2.96	67.89 ± 1.67	324.85 ± 9.75	73.73 ± 1.33
Sulfate-sulfur (S)	179.49 ± 4.48	101.89 ± 4.27	229.1 ± 4.58	160.84 ± 5.63
Chloride (Cl)	Not detected	Not detected	Not detected	Not detected
Potassium (K_2O)	< 1,000.0	< 1,000.0	< 1,000.0	< 1,000.0
Calcium (Ca)	468.32 ± 8.89	415.61 ± 4.98	591.05 ± 10.63	509.03 ± 7.64
Magnesium (Mg)	130.01 ± 1.82	116.73 ± 2.33	155.44 ± 3.73	131.62 ± 1.65
Iron (Fe)	26.96 ± 0.62	20.15 ± 0.96	36.44 ± 1.09	31.07 ± 0.34
Manganese (Mn)	5.53 ± 0.27	1.43 ± 0.03	10.25 ± 0.41	5.97 ± 0.07
Boron (B)	16.86 ± 0.17	15.35 ± 0.42	17.06 ± 0.22	15.22 ± 0.08
Copper (Cu)	2.12 ± 0.13	2.04 ± 0.06	3.01 ± 0.06	2.62 ± 0.02
Molybdenum (Mo)	Not detected	Not detected	Not detected	Not detected
Zinc (Zn)	18.53 ± 0.92	13.74 ± 0.35	23.65 ± 1.18	13.92 ± 0.07
Nickel (Ni)	Not detected	Not detected	Not detected	Not detected
Cobalt (Co)	Not detected	Not detected	Not detected	Not detected

ppm = parts per million.
values are presented as mean ± SE.

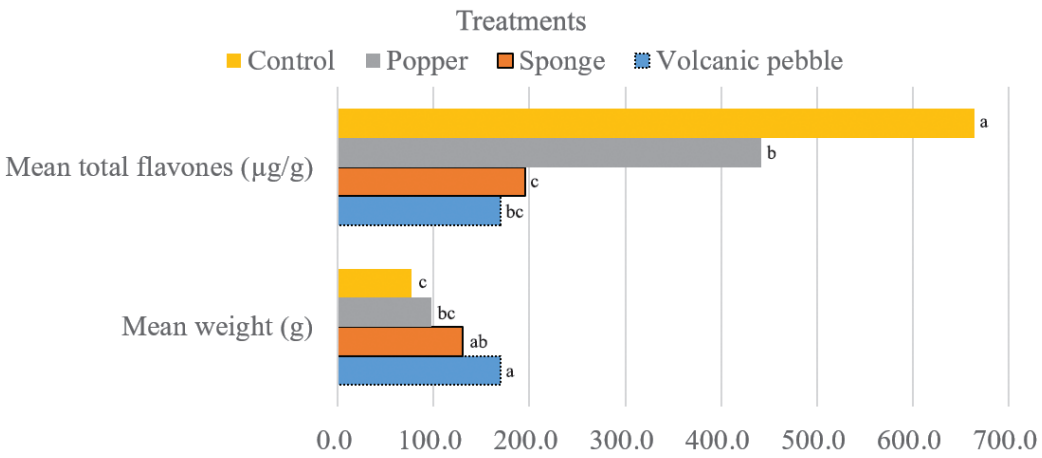


Fig. 2 Extracted total flavone contents and the average yield of black galingale dried rhizomes harvested after 14 d of growth in different media, where bars with different letters are significantly different ($p < 0.05$).

sponge treatment was the second highest at 130.58 ± 19.68 g/bucket (equivalent to 5,223.2 kg/ha). This was not significantly different from the pebble and popper treatments and only differed significantly from the control. The yield from the popper treatment was 97.92 ± 8.86 g/bucket (equivalent to 3,916.8 kg/ha). This was higher than, but not significantly different from, the control. The yield from the control treatment was 76.58 ± 16.15 g/bucket (equivalent to 3,063.2 kg/ha) ($F = 5.30$; degrees of freedom, $df = 3, 15$; $p = 0.05$). The average yield of the Rom-klao variety from a conventional highland plantation was only 2,504.2 kg/ha. This variety was reported to have the highest active compounds but not the highest yield (Pojanagaroon, 2011). In addition, the total aggregation of the seven flavones is shown in Fig. 2. As can be seen, the mean of total flavones was highest in the control (664.12 ± 52.74 µg/g) and significantly different from the popper (442.02 ± 57.93 µg/g), volcanic pebble (399.40 ± 45.62 µg/g) and sponge treatments (195.73 ± 27.96 µg/g). The mean total flavones from popper were significantly different from sponge but not significantly different from volcanic pebble ($F = 16.39$; $df = 3, 8$; $p = 0.05$).

Comparison of active compounds

A special characteristic of *K. parviflora* is its flavonoid content. These compounds give it a marketable value, which differs from other rhizome products; however, due to its characteristic taste, black galingale cannot be consumed directly as a regular food and is either processed into wine or used as a medicinal herb (Nagahara et al., 2002). Furthermore, it is difficult to design a growth regime for *K. parviflora* that achieves both high yield and high amounts of active compounds (Pojanagaroon, 2008; Pojanagaroon and Kaewrak, 2009). The peaks of flavonoid extracted from *K. parviflora* from volcanic pebble treatment and standard chemicals are shown in Fig. 3A and 3B, respectively. Peak numbers 1, 8, 10, 11 and 12 were not identified because of the lack of standard chemicals available and the limited availability of instruments for structural elucidation. Some known flavone backbones modified from Tokiwa Phytochemical Co. Ltd. (2017) and some additional unidentified flavones from the current experiment are illustrated in Fig. 4. A comparison of the flavonoid contents among the treatments is shown in Fig. 5. Surprisingly, most flavonoid contents from the control treatment were higher and significantly different from all other treatments. The concentration of 5,7,3′,4′-tetramethoxyflavone in the control was the highest among the treatments; however, this compound in the volcanic pebble and popper treatments was higher and significantly different from the sponge treatment ($F = 21.55$; $df = 3, 8$; $p = 0.05$). Similar results were obtained for the other six compounds: 3,5,7,3′,4′-pentamethoxyflavone ($F = 13.12$; $df = 3, 8$; $p = 0.05$), 5,7-dimethoxyflavone ($F = 14.95$; $df = 3, 8$; $p = 0.05$), 5,7,4′-trimethoxyflavone ($F = 22.12$; $df = 3, 8$; $p = 0.05$), 3,5,7-trimethoxyflavone ($F = 15.8$; $df = 3, 8$; $p = 0.05$),

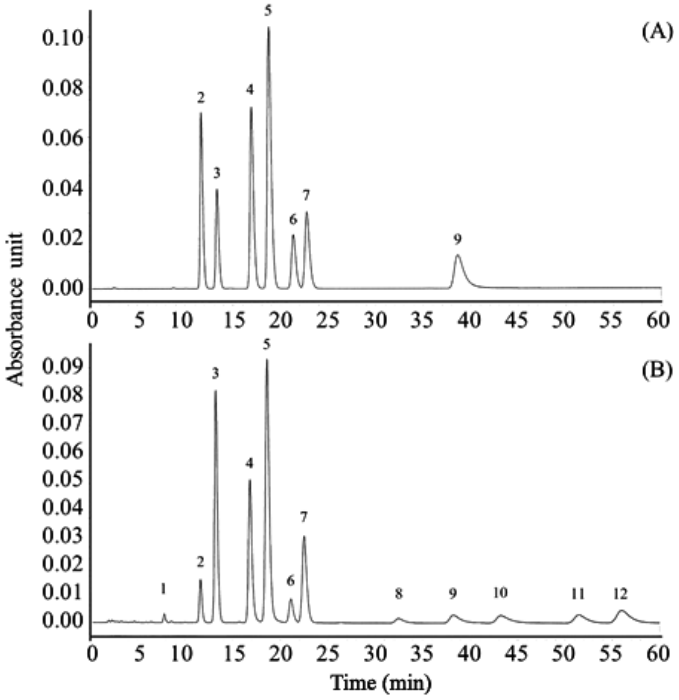
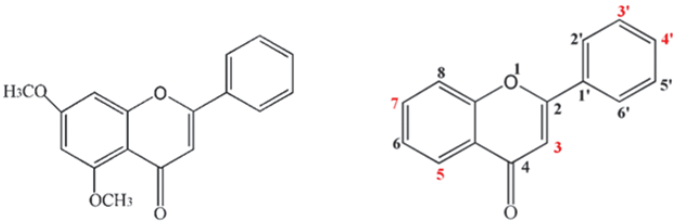


Fig. 3 Peaks of flavone analogs: (A) spectrum illustrating eight known standards: 5-hydroxy-7-methyflavone (#2), 5,7-dimethoxyflavone (#3), 4',5,7-trimethoxyflavone (#4), 3',4',5,7-tetramethoxyflavone (#5), 3,5,7,3',4'-pentamethoxyflavone (#6), 3,5,7-trimethoxyflavone (#7) and 3,5,7,4'-tetramethoxyflavone (#9); (B) spectrum illustrating peaks found in black galingale extracts grown in volcanic pebbles, where peaks # 1, 8, 10, 11 and 12 are unknown



Peak no.	Identified constituent	Substitutional groups				
		3	5	7	3′	4′
1	Unknown					
2	5-hydroxy-7-methoxyflavone		OH	OMe		
3	5,7-dimethoxyflavone		OMe	OMe		
4	4',5,7-trimethoxyflavone		OMe	OMe		OMe
5	3',4',5,7-tetramethoxyflavone		OMe	OMe	OMe	OMe
6	3,5,7,3',4' -pentamethoxyflavone	OMe	OMe	OMe	OMe	OMe
7	3,5,7-trimethoxyflavone	OMe	OMe	OMe		
8	Unknown					
9	3,5,7,4′-tetramethoxyflavone	OMe	OMe	OMe		OMe
10	Unknown					
11	Unknown					
12	Unknown					

Fig. 4 Backbone structure of known and unknown flavones (Tokiwa Phytochemical Co. Ltd., 2017), where O = Oxygen atom and Me = Methyl group.

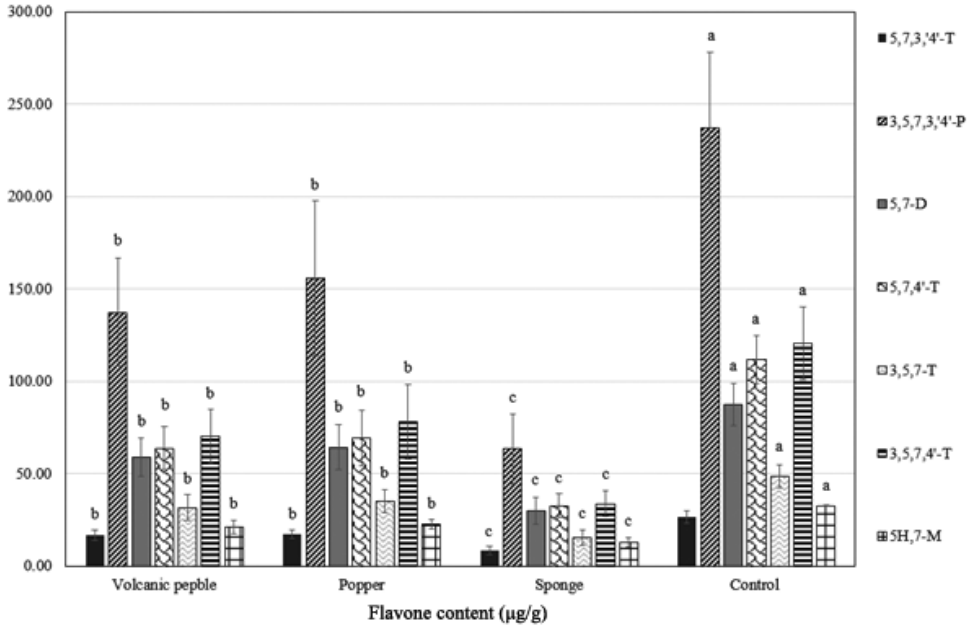


Fig. 5 Histograms showing mean content of seven flavones in black galingale rhizome grown in different potting systems, where 5,7,3′,4′ -T = 5,7,3′,4′-tetramethoxyflavone; 3,5,7,3′,4′-P = 3,5,7,3′,4′-pentamethoxyflavone; 5,7-D = 5,7-dimethoxyflavone; 5,7,4′-T = 5,7,4′-trimethoxyflavone; 3,5,7-T = 3,5,7-trimethoxyflavone; 3,5,7,4′- = T = 3,5,7,4′-tetramethoxyflavone; 5H,7-M = 5-hydroxy-7-methoxyflavone; different lowercase letters above bars indicate significant differences ($p < 0.05$) and error bars indicate ± SE.

3,5,7,4′-tetramethoxyflavone (F = 14.53; df = 3, 8; $p = 0.05$) and 5-hydroxy-7-methoxyflavone (F = 29.91; df = 3, 8; $p = 0.05$). These results might have been due to the lack of supporting material in the control treatment to adsorb the nutrients, thus allowing maximum absorption by the black galingale root, while some ion portions were adsorbed by the media in the other treatments. Since 5,7-dimethoxyflavone and 5,7,4′-trimethoxyflavone are deemed to be the major components of *K. parviflora* rhizomes, they have become an index for commercial trading (Sutthanut et al., 2007). In contrast, the results from the current study differed, with 3,5,7,3′,4′-pentamethoxyflavone being the most abundant compound in every treatment. In this study, the proportions of each flavone were similar across treatments, merely differing in concentrations. However, in order to increase the concentration of any specific flavone, both the growing conditions and nutrients will require further investigation.

Potting system

The only product harvested was the popper treatment in the nylon net house, as all other treatments in the nylon net house and in the air-conditioned room died after the third month. The reason for this phenomenon was the outbreak of the two plant pathogens reported earlier. The mean (± SE) dried weight from the popper treatment was 98.7 ± 12.24 g/pot (equivalent to 3,948.0 kg/ha).

As mentioned earlier, most crop cultivation using soilless culture has emphasized plant growth and yield, and no studies have proposed soil-free crop cultivation systems that produce high contents of active compounds (Suhaimi et al., 2012). Several planting materials including perlite, coconut coir, and peat moss were also preliminarily tested in both hydroponic and potting systems prior the experiment. The preliminary results was found that those materials degraded rapidly, as after the fourth month of application (data not shown). The frequent refilling required for these materials would not be economically feasible, and peat moss was found to easily clog the fertigation system. *K. parviflora* grew well in pots, but the high water content of the substrate might affect the air-filled porosity, thus limiting oxygen availability (Humara et al., 2002; Wall and Heiskenen, 2003). This condition might also have played a role in the infection by the two plant pathogens reported earlier (Burrage, 1992; Whipps, 1992). Therefore, the potting system is not suitable for growing *K. parviflora* unless appropriate media and an effective plant disease control method are available. However, the hydroponic system is worthy of further investigation since it is neither seasonal nor climate-dependent. Analyses of nutrient uptake should be done more frequently to determine which elements play a major role in the synthesis of active compounds. Disease prevention methods using microbial control agents (Maketon et al., 2008; Maketon et al., 2010) with optimized nutrient proportions and concentrations would help to achieve not only high yields but also high contents of several active compounds.

In conclusion, this experiment showed that volcanic pebble provided the best combination for growing *K. parviflora* in a hydroponic system when considering both yield and the total amount of active ingredients.

Conflict of Interest

The authors declare there are no conflicts of interest.

Acknowledgements

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