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Preliminary study of colorimetric detection for potential medicinal compounds of *Kaempferia parviflora* using phytochemical determination

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រុក្ខជាតិឱសថត្រូវបានប្រើប្រាស់ជាឱសថបុរាណដើម្បីការបង្ការ និងព្យាបាលជម្ងឺបានច្រើនមុខ ដែលជាការយល់ដឹង ឬហាត់រៀនតៗគ្នាតាំងពីបុរាណកាលមក។ *K. parviflora* គឺជារុក្ខជាតិម្យ៉ាងដែលមានប្រវត្តិដ៏យូរលង់ណាស់មកហើយ និងមានលក្ខណៈសម្បត្តិជាច្រើនក្នុងការប្រើប្រាស់ជាឱសថ។ គោលបំណងបឋមនៃការសិក្សាស្រាវជ្រាវនេះ គឺផ្តោតសំខាន់ទៅលើរុក្ខជាតិឱសថបុរាណ *K. parviflora* ដែលត្រូវបានគេប្រើប្រាស់សម្រាប់ព្យាបាលជំងឺលើសសម្ពាធឈាម ព្រមទាំងជួយឱ្យមានសុខភាពល្អនិងអាយុវែង។ លទ្ធផលនៃការសិក្សានេះបានបង្ហាញថា *K. parviflora* មានសមាសធាតុឱសថល្អៗដូចជា flavonoid, phenolic, tannin និង terpenoid ។ ការអង្កេតជាបឋមបានបង្ហាញថា សារធាតុពណ៌ដែលចំរាញ់ចេញពី *K. parviflora* បានផ្តល់ទំនុកចិត្តខ្ពស់ក្នុងការវាយតម្លៃជាវិជ្ជមានលើការប្រើប្រាស់ឱសថបុរាណនេះ។ ការអង្កេតនេះបានបង្ហាញថា ការជ្រើសរើសយកការអង្កេតជាបឋមទៅលើសារធាតុពណ៌គឺអាចទទួលយកបាន។ ប៉ុន្តែទោះជាយ៉ាងនេះក្តី ការប្រើក្រូម៉ាតូក្រាហ្វីក្នុងការវិភាគកំហាប់គីមីគឺជារឿងចាំបាច់បំផុតសម្រាប់ការសិក្សាបន្ថែម។

ABSTRACT

Medicinal plants are used in traditional medicine to treat various diseases. They have been utilized to prevent and cure numerous diseases, the treatment of which is established in conventional knowledge practices. *K. parviflora* is a perennial plant with numerous medicinal properties. This study seeks to conduct a preliminary investigation of traditional medical treatment using *K. parviflora*, which has been employed for treating hypertension and promoting longevity through overall good health. In its results, this study shows that *K. parviflora*

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contains potential medicinal compounds such as flavonoids, phenolics, tannins, and terpenoids. The preliminary colorimetric detection of the *K. parviflora* extracts provides high confidence in the positive assessment of traditional medical practice. The present investigation suggests that using colorimetric detection for initial screening is acceptable. However, it is strongly recommended that chromatography be used in chemical concentration analysis for further study.

1. Introduction

Kaempferia parviflora (hereafter referred to as *K. parviflora*) is known as Black ginger, a plant species belonging to the Zingiberaceae family, which comprises about 53 genera and over 1200 species in total (Kress et al., 2002). It is established knowledge that normal ginger (*Zingiber officinale*) and *K. parviflora* have similar morphological characteristics to rhizomes, but their chemical properties are different (Asamenew, et al., 2019). According to its classification, *K. parviflora* has a chromosome number of 2n = 22 (Nopporncharoenkul et al., 2017), and it belongs to the ginger family, and its binomial name is *Kaempferis parviflora* Wall. ex Baker (Sirirugsa, 1991) (**Table 1**). The *K. parviflora* is a vascular plant in the tracheophytes group, a flowering plant (Wasuntarawat et al., 2021; Tewtrakul et al., 2008), and its life cycle is almost 225 days. The *K. parviflora* plant has deep purple-colored rhizomes and is originally native to the North and Northeast of Thailand (Nopporncharoenkul et al., 2017; Saokaew, et al., 2017). It is widely distributed in tropical regions and is taxonomically characterized as perennial, aromatic, and tuberous, with non-tuberous rhizomes (Jatoi et al., 2007). Its rhizome is the plant's main part, which is economically significant and offers a rich source of effective phytoconstituents for biological activities (Wu & Larsen, 2000). Moreover, it has been used as a medicinal resource for treating various diseases (Fathy et al., 2015). For the most part, in Cambodia, it is not known that *K. parviflora* is a medicinal plant and can be used for health purposes, even though in the scientific community, it has been the topic of increased interest in recent years. Nevertheless, in Thailand, it has been known as Thai ginseng, and its rhizome has been used in folk medicine for many years (Tewtrakul et al., 2008).

Due to its wide range of functional health benefits, the *K. parviflora* has been used as a traditional remedy to treat different illnesses, particularly in traditional Thai medicine for centuries. It has many pharmaceutical applications (Yoshino et al., 2019; Mekjaruskul et al., 2012). Its rhizomes are also known as health-promoting herbs and, for a long time have been used in traditional medicine for managing a variety of diseases, including inflammation, ulcers, gout, colic disorder, abscesses, allergies, and osteoarthritis (Saokaew et al., 2017; Toda et al., 2016). Moreover, recent evidence demonstrates that *K. parviflora* has anticancer properties, also called an antineoplastic drug, that is effective in treating malignant or cancerous tumors (Suradej et al., 2019). The *K. parviflora* extract prevents the initiation of cancer cell activities (Horikawa, et al., 2012), provides cardioprotective properties (Yorsin et al., 2014), neuroprotective properties (Youn et al., 2016), and antioxidant and anti-inflammatory properties (Chen et al., 2018). Further studies have found that *K. parviflora* is a unique medicinal herb with an extensive range of pharmacological impacts serving to provide antioxidants and reduce inflammation, allergies, ulcers, viruses, bacterial infections, depression, and cancer, as well as cardio benefits, lowering cholesterol, combating Alzheimer's and supporting neurological health (Plaingam, et al., 2017; Weerapol et al., 2017). Although more recently addressed in ethnopharmacological studies, its rhizome has long been used as traditional medicine among indigenous groups to improve their physical stamina, promote overall health, and increase longevity or life expectancy. According to one previous study, *K. parviflora* has been used in traditional medicine to enhance sexual performance (Temkitthawon et al., 2011).

Table 1. Scientific classification of *K. parviflora* Wall. ex Baker (Zingiberaceae). Adapted from (Sirirugsa, 1991).

Kaempferia parviflora Wall. ex Baker (Zingiberaceae)

Commonly known	Scientific classification	
Kaempferia parviflora	Kingdom:	Plantae
Black ginger	Phylum:	Tracheophytes
Thai black ginger	Class:	Monocotyledon
Thai ginseng (krachai dum)	Order:	Zingiberales
	Family:	Zingiberaceae
	Genus:	Kaempferia
	Species:	*parviflora*
	Binomial name:	*Kaempferia parviflora*

Currently, *K. parviflora* products are marketed as dietary supplements for enhancing nutrition, in the form of capsules,

in certain countries such as Thailand and Japan. Among the various phytochemical components of the K. parviflora rhizome are polymethoxflavones, including 5,7-dimethoxflavone, 5,7,4'-trimethoxyflavone, 3,5,7,3',4'-pentamethoxyflavone, 5-hydroxy-3,7-dimethoxyflavone and 5-hydroxy-3,7,4'-trimethoxyflavone (Gopi et al., 2017; Sutthanut et al., 2007). The biological effects of the major flavonoids, phenolic, tannins, and terpenoids in *K. parviflora* rhizomes have not yet been clarified through research studies. However, the beneficial results of these compounds against degenerative processes associated with oxidative stress have been corroborated through research and reported (Silberberg, et al., 2005). Prior studies have identified the phytochemical compositions of flavonoids, phenolics, tannins, and terpenoids by using techniques such as gas chromatography-mass spectrometry (GC-MS) (Sagbo et al., 2022; Willie et al., 2021), high-performance liquid chromatography (HP-LC) (Olasunkanmi et al., 2022), molecular spectroscopy (UV-Vis and FTIR), hyphenated chromatography (UHPLC-qTOF-MS) (Mabasa et al., 2021), and high-performance liquid chromatography with diode-array detection (HP-LC-DAD) (Falode et al., 2019). However, those techniques are both costly and relatively time-consuming (Dimzoska et al., 2022). At the same time, chromatography techniques are helpful in precisely separating, analyzing, and purifying a wide range of samples (D'Atri et al., 2018).

In more recent investigations, colorimetric detection has been a frequently used technique as a primary indicator and can be utilized under ambient conditions, entails low-cost physics experiments, is environmentally friendly and easy to apply, and provides for precise and rapid detection (Sun et al., 2021). Colorimetry is a solution-based assay that is used to determine the concentration of colored compounds and to estimate the concentration of a solution by measuring its absorbance at a suitable wavelength (Gopinath et al., 2014). This paper-based technique enables effective colorimetric detection of different types of chemical compounds. This detection method has the advantage of rapidity and simplicity over other approaches. This study utilized this low-cost and novel colorimetric measurement technique to detect flavonoid, phenolic, tannin, and terpenoid compounds, which can potentially be used for medicinal purposes. This study aimed to effectively screen and determine the existence of such compounds in *K. parviflora*, allowing for a determination of the potential for their extraction and medical applications.

2. Material and methods

2.1 Chemicals and plant material

In this study, the chemicals used in research techniques included ethanol (C_2H_5OH), dichloroethane ($C_2H_4Cl_2$), methanol (CH_3OH), sodium hydroxide (NaOH), ferric chloride ($FeCl_3$), sulfuric acid (H_2SO_4), and chloroform ($CHCl_3$). They were purchased from Sigma-Aldrich, based in the USA. All chemicals were analytical grade. For plant materials, the fresh rhizomes of 20 kg of *K. parviflora* were collected from different villages in Anlong Thom commune, located in Preah Jayavarman-Norodom Phnom Kulen National Park, which is in the province of Siem Reap (**Fig.1**).

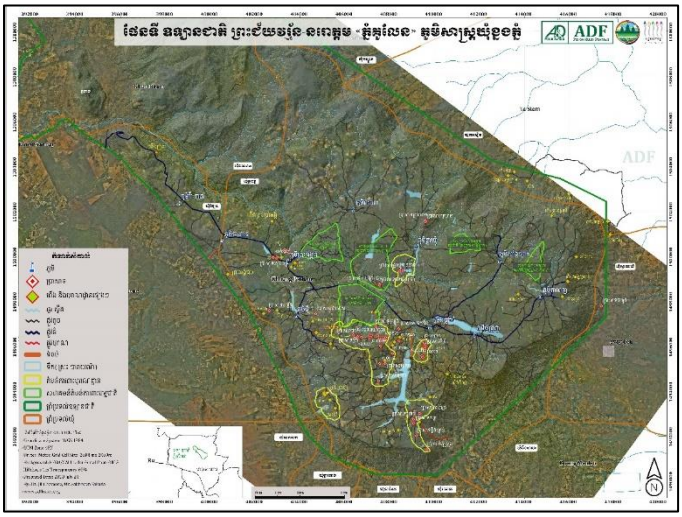

Fig. 1. Map of Anlong Thom commune, Preah Jayavarman-Norodom Phnom Kulen National Park, Siem Reap province (Source: Archaeology and Development Foundation, ADF).

2.2 Preparation of *K. parviflora* extracts

In preliminary screening, the fresh rhizomes of *K. parviflora* were cleaned twice with distilled water and then soaked in a solution containing 75% ethanol for 5 minutes to disinfect surfaces to remove contamination. After the practice of disinfecting the samples, the rhizomes were cut into small pieces and then dried in the incubator at 75°C for 72 hours. The dried rhizomes were turned into powder by using a blender machine. The dried powder was kept at 4°C in a refrigerator until the experiment was conducted. Regarding crude preparation, 10 g of the dried powder was mixed with 100 mL dichloroethane and 100 mL methanol. Then, it stirred for 3 minutes five times every 2 hours. After that, the solution was aseptically filtered using filter paper (Whatman® No.1 brand). The filtrate solution was then evaporated to form a crude substance. The screening process for chemical compounds was carried out according to prior studies (Riaz et al., 2015; Pasto & Johnson, 1979). Distilled water was used for a control reaction during the preliminary screening. The experiments were performed in triplicate (n = 3) and repeated three times, as there were three independent control reaction tests.

2.3 Determining tannin content

A ferric chloride test was used to determine the presence of tannin compounds in the crude solution and was performed following the process elaborated by Pasto and Johnson (1979). In brief, 25 mg of the crude substance was boiled with 5 mL of distilled water. Then, it was filtered utilizing filter paper (Whatman® No. 1). The filtrated solution was kept at ambient temperature (room temperature) until it reached that temperature. Then, 150 µL of $FeCl_3$ (0.1% *w*/*v*) was added to the filtrate. The dark-brown coloration demonstrated the presence of tannin after the reaction.

2.4 Determining phenolic content

The ferric chloride test used to indicate the presence of phenolic compounds in the crude solution was also performed following Pasto and Johnson (1979). In brief, the crude substance of 25 mg was dissolved in 2 mL of a solution

composed of 95% ethanol. Afterward, 150 µL of $FeCl_3$ (0.5% *w*/*v*) was added to the solution. In the results, the presence of phenolic compounds was observed by the appearance of a bluish-black color.

2.5 Determining flavonoid content

The flavonoid test indicates the presence of flavonoids in the crude, as Riaz et al. (2015) reported. Shortly, the crude substance of 25 mg was dissolved in 2 mL of a solution composed of 95% ethanol. Then, 150 µL of NaOH (10% *w*/*v*) was added to the solution to create a yellow color. After that, 1 mL of conc. H_2SO_4 was added to the solution. After adding H_2SO_4, the presence of flavonoids was observed by the disappearance of the yellow color and the appearance of the solution turning to a dark brown color.

2.6 Determining terpenoid content

The terpenoid test was used to indicate the presence of terpenoids in the crude, as established by Riaz et al. (2015). Concisely, the crude substance of 25 mg was dissolved in 2 mL of $CHCl_3$. At that time, an amount of 150 µL of concentrated H_2SO_4 was added to the solution to form two layers in the solution. As in a speedy reaction, the presence of terpenoids was indicated by the formation of a reddish-brown color at the interface between the two layers.

3. Results and discussion

3.1 Preliminary phytochemical screening of the tannin, phenolic, flavonoid, and terpenoid

The preliminary phytochemical screenings of tannin, phenol, flavonoid, and terpenoid in *K. parviflora* were observed based on the color in the solution, which appeared upon adding the reagent. The different reagents and extraction solvents were used to distinguish the other compounds by observing color complex formation. The experiments were conducted to screen for the presence of the components mentioned above, shown in **Table 2**. A dark-brown coloration demonstrated the presence of tannins after resection. The presence of phenolic compounds was detected by the appearance of a bluish-black color in the final reaction.

The presence of terpenoids was also indicated by the formation of a reddish-brown color at the interface between the two layers of the solution. In the ferric chloride test, the neutral ferric reacts with the phenol functional group resulting in complexation which generates blue, green, violet, red, or mixed colors, according to the presence of different derivatives of phenolic compounds (Klangmanee & Athipornchai, 2019). However, the ferric chloride test is not specified as the only means to determine the presence phenol functional group, as the tannins also provide positive signs due to the phenolic functional group of the tannin compounds.

In the experiment, the tannins and phenolic compounds were tested using different concentrations of ferric chloride and other solvent extraction processes, which led to different resulting colors. The result suggests that heat extraction using water gives a more concentrated tannin solution, and only 0.1% (*w*/*v*) of ferric chloride could turn the solution brownish. The alcohol functional group can also explain this on the aromatic ring, which could make the compound more water soluble as well as a complex with ferric ions.

The results of the ferric chloride test are corroborated by the results of the previous work (Abdelfatah et al., 2021; Eslami et al., 2018). The presence of flavonoids is indicated by reacting the organically extracted crude with the base, followed by the formation of a yellowish color, then adding concentrated sulfuric acid and observing the resulting color as dark brown. Using ethanol extraction, most of the flavonoids in the crude could be detected. However, results could also be observed to show terpenoids (Sitthichai et al., 2022; Riaz et al., 2015). Therefore, adding base could create a reaction with flavonoid compounds resulting in a yellow color before adding sulfuric acid. A nonpolar solvent (dichloromethane) was used to indicate terpenoids, and the extracted compounds were reacted with concentrated sulfuric acid directly. The terpenoid compounds would be complex with acid and generate a reddish-brown color at the interface between the double layers. These phytochemical screening methods could distinguish and indicate the presence of flavonoids and terpenoids in the crude derived from *K. parviflora*.

The *K. parviflora* is a medicinal plant in the family Zingiberaceae. Its rhizome has been used as traditional medicine for many centuries. According to the anecdotal evidence of safety and efficacy, *K. parviflora* has been selected in Thailand as 1 of the five most beneficial herbal products that have been widely used and have generated income for the country. Several pharmacological studies of *K. parviflora* have found benefits for various illnesses (Saokaew et al., 2017). Plant-based alternative medicinal products are of significant interest due to their extensive availability and fewer adverse effects. Aimed at the characteristics of a particular substance, tannins are polyphenolic compounds. They have been found to possess protective benefits, including anti-inflammatory properties, anti-fibrotic properties, anti-microbial properties, anti-diabetic properties, and anti-cancer properties, among others (Rajasekar, et al., 2021). Designed phenolic compounds play an essential role in regulating health and disease. The presence of phenolic compounds in *K. parviflora*, in addition to their bioavailability and protective effects, is of interest for treating diabetes mellitus. Moreover, phenolic compounds can inhibit enzymes associated with the development of human diseases and have been used to treat various common human ailments, including hypertension, metabolic problems, incendiary infections, and neurodegenerative diseases (Rahman, et al., 2021).

Recent years have seen an exponential expansion in the interest in flavonoids as dietary bioactive to prevent human diseases and in their candidacy as pharmaceutics to function as lead compounds. Flavonoids are a sub-class of plant polyphenols that have been shown to possess numerous health-promoting physiological benefits in a wide range of investigations, from cell-based assays to epidemiological and human intervention studies. Beyond their role as biologically active molecules found in plant-based foods, new insights have arisen into the potential use of flavonoid derivatives as effective therapeutics to manage certain cancers. Flavonoids also offer promising applications in managing obesity and

Table 2. Color reaction experiments to detect flavonoids, phenolics, tannins, and terpenoids.

Investigated compound	Reaction conditions		Color reaction test
Tannin	Control	Distilled water 25 µL + Distilled water (5 mL) + Ferric chloride (0.1%, *w*/*v*) 150µL	
	Sample	Crude 25 mg + Distilled water (5 mL) + Ferric chloride (0.1%, *w*/*v*) 150 µL	
Phenolic	Control	Distilled water 25 µL + Ethanol 95% (2 mL) + Ferric chloride (5%, *w*/*v*) 150 µL	
	Sample	Crude 25 mg + Ethanol 95% (2 mL) + Ferric chloride (5%, *w*/*v*) 150 µL	
Flavonoid	Control	Distilled water 25 µL + Ethanol 95% (2 mL) + NaOH (10%, *w*/*v*) 150 µL + Sulfuric acid (1 mL)	
	Sample	Crude 25 mg + Ethanol 95% (2 mL) + NaOH (10%, *w*/*v*) 150 µL + Sulfuric acid (1 mL)	
Terpenoid	Control	Crude 25 mg + Chloroform (2mL) + Sulfuric acid (150µL)	
	Sample	Distilled water 25 µL + Chloroform (2 mL) + Sulfuric acid (150 µL)	

inflammation-associated disorders and controlling infectious diseases, including coronavirus (Rupasinghe, 2020).

In pharmacological activities, terpenoids possess antitumors, anti-inflammatory, antibacterial, antiviral, and antimalarial effects, promote transdermal absorption, prevent and treat cardiovascular diseases, and have hypoglycemic effects. In addition, terpenoids have many potential applications, such as immunoregulation, antioxidation, antiaging, and neuroprotection (Yang et al., 2020). The following effects of *K. parviflora* has been found in clinical testing or trials: antiallergenic, anti-inflammatory, antimutagenic, anti-depressive, anticholinesterase, antimicrobial, anticancer, anti-peptic ulcer, cardioprotective, anti-obesity, and aphrodisiac (Azuma et al., 2011; Akase et al., 2011; Malakul et al., 2011; Banjerdpongchai, et al., 2008; Sudwan et al., 2006; Rujjanawate, et al., 2005). Other studies have also described the considerable medicinal utility of *K. parviflora* (Begum et al., 2022).

4. Conclusion

In this study, we use the phytochemical screening method to indicate the presence of flavonoids, phenolics, tannins, and terpenoids to demonstrate the properties that could provide for potential medicinal applications of *K. parviflora*. According to our findings, *K. parviflora* contains bioactive compounds that show the reasonableness of the established traditional use of *K. parviflora* in conventional medicine practices. However, even though it has been widely used for a long time and has been known to have benefits, pharmaceutical studies in Cambodia on the plant still need to be expanded. Such a study could open the door to further identifying or quantifying the presence of active compounds of the species *K. parviflora* in Cambodia. In the present investigation, the chemical constituents isolated from rhizomes of *K. parviflora* must be subjected to further tests to determine the measurement of the concentration of the components. Phytochemical screening is the primary step for further investigating the quantity and quality of ingredients known to have health benefits of the *K. parviflora* that is grown in Cambodia.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported. All authors have read and approved the final, published version of the manuscript.

Credit authorship contribution statement

OURN Eneang: Experimental design, Laboratory experiments, Data interpretation, Writing-review & editing. LY Viboth: Experimental design, Data interpretation, Supervision, Funding acquisition, Writing-review & editing. POL Thev: Data interpretation, Writing-review & editing. CHEM Chanchao: Conceptualization, Experimental design, Data interpretation, Visualization, Writing-original draft, Writing-review & editing.

All authors have read and agreed to the published version of the manuscript.

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