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Alpinia nelumboides Nob.Tanaka, T.T.K.Van & V.Hoang: phytochemical analysis and antioxidant activities of pseudo-stem and rhizome essential oils

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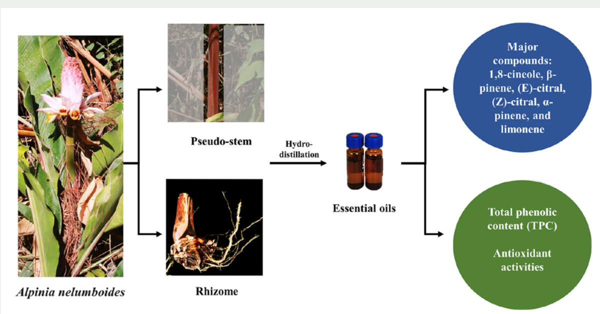
ABSTRACT

Alpinia nelumboides Nob.Tanaka, T.T.K.Van & V.Hoang is the new *Alpninia* species discovered in Vietnam in 2023. Herein, we first hydrodistillated its pseudo-stems and rhizomes to obtain its essential oils, PS-EO and RH-EO. Their volatile compounds and total polyphenols were analysed by gas chromatography-mass spectrometry and the Folin-Ciocalteu method, respectively. Antioxidant activities were determined using four different approaches. The results showed that PS-EO and RH-EO contained 40 and 31 compounds, accounting for 99.78% and 99.45% of their compositions, respectively. The contents of polyphenols and monoterpenes in PS-EO were higher than in RH-EO. RH-EO displayed weaker scavenging activities (17.40–19.53%) than PS-EO (30.81–44.08%). PS-EO also showed higher ferric and cupric reducing powers, with EC_{50} values of 3.50–5.30 mg/mL smaller than RH-EO's EC_{50} values of 19.0–23.0 mg/mL. These results first revealed the phytochemical profile and antioxidant activities of EOs from *A. nelumboides*.



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1. Introduction

The genus *Alpinia*, a member of the Zingiberaceae family, is of great significance as it encompasses approximately 250 species that are predominantly found in India, Malaysia, China, Australia and the Pacific Islands (Zhang et al. 2016). Many *Alpinia* plants have been applied to Asian cuisine or traditional medicine for centuries. For instance, *A. galanga* is an important component of curries and is widely used as a flavouring agent for meats and soups in Southeast Asia (Yang and Eilerman 1999; Cheah and Abu Hasim 2000). *A. oxyphylla* Miquel is traditionally used in China to treat diarrhoea, abdominal pain and intestinal disorders (Zhang et al. 2013; Yu et al. 2020).

Previous studies have demonstrated that essential oils (EOs) obtained from *Alpinia* species exert multiple bioactivities of importance to human health. For example, EOs from *A. calcarata* leaves and rhizomes and their major chemical compounds (1,8-cineole, *α*-terpineol and fenchyl acetate) inhibited the release of proinflammatory mediators such as nitric oxide, reactive oxygen species (ROS), prostaglandins, cyclooxygenases and cytokines induced by lipopolysaccharides in murine macrophages (Chandrakanthan et al. 2020). They also alleviated oedema and pain in a mouse study of inflammation and pain.

Alpinia nelumboides (Figure S1), a new species from Champasak province, southern Laos and Lam Dong province, southern Vietnam, was described for the first time in 2022 (Tanaka et al. 2023). The plant, locally known as 'Kha Dok Bua' in Laos or 'Riềng sen' in Vietnam, is a perennial herb with thick, hard creeping rhizomes (3–4 cm in diameter). In terms of morphological characteristics, the shape of the inflorescence and flower colouration, *A. nelumboides* exhibits the greatest resemblance to *A. kwangsiensis.* Its flowers (5.5–7 cm long) are pink or white in colour, with tubular calyxes (2.5–2.8 cm long) (Tanaka et al. 2023). Previous research has indicated that the EOs of several commonly found *Alpinia* species consist of various major constituents, including 1,8-cineole, pinenes, camphene, terpinen-4-ol, α-terpineol and fenchyl acetate (Padalia et al. 2010; Dai et al. 2020; Nhan et al. 2021). For example, in A. galanga rhizome oil, 1,8-cineole could make up 42.3%, while the total percentage of pinenes broadly ranged from 1–65%.

Oxidative stress, which is caused by the overproduction of reactive oxygen species (ROS), is linked to the pathogenesis of various diseases, such as neurodegenerative diseases and cancer (Schieber and Chandel 2014; Porres-Martínez et al. 2016). Phytochemicals and EOs from medicinal plants can be incorporated to regulate cellular redox balance, thus preventing ROS from oxidising biomolecules (Porres-Martínez et al. 2015). Indeed, several EOs from *Alpinia* species such as *A. galanga* collected in Thailand or *A. officinarum* originated from China have shown broad ranges of antioxidant activities against free radicals, in which 1,8-cineole, 4-allyphenyl acetate, *β*-bisabolene, *α*-pinene and *β*-pinene were considered the main active compositions (Tachakittirungrod and Chowwanapoonpohn 2007; Zhang et al. 2010).

To our knowledge, no data about the chemical composition of EOs from *A. nelumboides* have been reported in the literature. In this study, we analysed the volatile profile, total polyphenols and antioxidant activities of EOs extracted from the pseudo-stem and rhizome of the plant. The findings provide the first evidence of the bioactive constituents in EOs of this species as well as its potential applications.

2. Results and discussion

2.1. Chemical composition of the EOs

Hydrodistillation of *A. nelumboides* pseudo-stems and rhizomes produced light yellow EOs with yields of 0.04% and 0.13% (w/w) (based on the fresh weight of samples), respectively. Gas chromatography-mass spectrometry (GC/MS) analysis (Figures S2 and S3) indicated 40 and 31 compounds in the pseudo-stem EOs (PS-EO) and rhizome EOs (RH-EO), accounting for 99.78% and 99.45% of the compositions, respectively (Table S1). The phytochemical compounds in EOs were oxygenated monoterpenoids (61.94% in PS-EO, 82.16% in RH-EO), monoterpene hydrocarbons (36.49% in PS-EO, 16.96% in RH-EO), sesquiterpene hydrocarbons (0.95% in PS-EO, 0.06% in RH-EO), oxygenated sesquiterpenes (0.34% in PS-EO, 0.20% in RH-EO) and non-terpenes (0.06% in PS-EO, 0.07% in RH-EO).

In general, PS-EO and RH-EO were rich in compounds, such as 1,8-cineole, *β*-pinene, (*E*)-citral, (*Z*)-citral, *α*-pinene and limonene. Specifically, the main chemical constituents of the PS-EO were 1,8-cineole (20.01%), (*E*)-citral (18.06%), *β*-pinene (17.52%), (*Z*)-citral (11.58%), *α*-pinene (10.31%), limonene (6.02%) and geranyl acetate (4.62%). Meanwhile, 1,8-cineole (48.62%), linalool (11.83%), (*E*)-citral (9.97%), (*Z*)-citral (6.04%), *α*-pinene (6.03%), limonene (4.51%) and *β*-pinene (4.10%) were the main chemical constituents of the RH-EO. These results are similar to a previous review, which summarised the chemical composition of EOs from several species of the *Alpinia* genus, showing that 1,8-cineole, *β*-pinene, *α*-pinene, *β*-myrcene, camphor, *γ*-terpinene, *p*-cymene, geraniol, *α*-fenchyl acetate, ocimene, methyl cinnamate and *β*-caryophyllene are the main constituents (Van et al. 2021).

PS-EO and RH-EO of *A. nelumboides* also showed variations in their compositions. Fenchyl acetate, intermedeol, bornyl acetate, *α*-terpinyl acetate, methyl thymyl ether and fenchol that occurred in small amounts in the rhizome oil were not present in the pseudo-stem sample. Similarly, *α*-phellandrene, *iso*-neral, citronellal, *α*-cubebene, citronellol acetate, copaene, *β*-elemene, humulene, aristolochene, *epi*-cubebol, *α*-farnesene, cubebol, *α*-epi-cadinol and (2*E*, 6*E*)-farnesal were detected in RH-EO but not in PS-EO. The rhizomes and pseudo-stem of *A. nelumboides* were from the same plant specimen, which was collected at the same time and extracted under similar conditions. Thus, the variance in volatile composition between RH-EO and PS-EO may be attributed to the different parts of the plant. This variation was also observed in the EOs extracted from *A. kwangsiensis* stems and rhizomes (Nhan et al. 2021) or the EOs from leaves and rhizomes of *A. conchigera* Griff, which showed some compounds occurred only in one type of EO and were not in the remaining one (Qamaruz Zaman et al. 2021).

Since *A. nelumboides* exhibits the greatest resemblance to *A. kwangsinesis*, the chemical compositions in the EOs of this new species were compared with those of *A. kwangsiensis* (Table S1). The results showed that despite sharing some main compounds like *α*-pinene, *β*-pinene, limonene and 1,8-cineole, the two *Alpinia* species volatile compositions were greatly different. Several compounds, such as pinocarvone, borneol, *p*-cymene-8-ol, (*E*)-methyl cinnamate, were only present in *A. kwangsinesis's* EO, while 5-hepten-2-one, 6-methyl-, linalool, *α*-terpineol, (*Z*)-citral, (*E*)-citral, geraniol and geranyl acetate were only found in *A. nelumboides's* EOs.

The amounts of monoterpenes and oxygenated monoterpenes in *A. nelumboides* EOs were also much higher than those in the EOs of *A. kwangsiensis.* These results demonstrated the differences in volatile compositions of *A. nelumboides* and *A. kwangsinesis,* a well-studied *Alpinia* species.

2.2. Total phenolic content (TPC)

TPC is widely considered the main bioactive, directly contributing to the antioxidant activity of plant extracts. There are several studies on total polyphenols in the EOs of rhizomes of different *Alpinia* species. However, the information about the TPC of *Alpinia*'s PS-EO has remained limited. The TPC of EOs from rhizomes and pseudo-stems were 0.48 ± 0.02 and 1.61 ± 0.04 mg GAE/g EO, respectively (Table S2). Compared with a previous study, the total polyphenols in EOs from *A. zerumbet* and *A. conchigera*, collected from the northeastern region of Thailand, were reported to be in the range of 1.86–1.99 mg GAE/g EO (Chumroenphat et al. 2019). These levels were quite similar to TPC in PS-EO, while TPC in RH-EO was approximately 3.3 times as small as that in pseudo-stem. Similarly to volatile composition, the variations in TPC of rhizomes and pseudo-stems could be due to the different elements of the studied plants, as other reports showed that there was a similar variance in the TPC in rhizome EO to leaf EO *(*Qamaruz Zaman et al. 2021*)* or in flower EO to seed EO *(*Elzaawely et al. 2007*)* of different *Alpinia* species across several countries.

2.3. Antioxidant activities

Due to complicated mechanisms, antioxidant activities are often determined using different approaches. In this study, we evaluated the antioxidant activities of EOs based on two main mechanisms: Free radical scavenging and metal-reducing powers. The EOs displayed concentration-dependent antioxidant activity, in which PS-EO exhibited higher antioxidant activities than RH-EO.

Regarding scavenging activities, the activities of both EOs ranged from 17.40–44.08%. Specifically, the DPPH scavenging activity of PS-EO (44.08%) at a concentration of 100 mg/mL was approximately 2.6 times as high as that of RH-EO (17.4%) (Figure S4). As for ABTS free radicals, the scavenging activities of RH-EO and PS-EO were 19.53% and 30.81%, respectively, at a concentration of 100 mg/mL (Figure S5). The scavenging activities of both EOs were much weaker than the positive control; The IC_{50} values of ascorbic acid against DPPH and ABTS free radicals—the concentration at which Ascorbic acid scavenges 50% free radicals—were 0.01 and 0.05 mg/mL, respectively (Table S3). However, these scavenging activities of PS-EO and RH-EO were similar to the range of activities of some other EOs. The percentage of DPPH scavenging activity of the EO extracted from rhizomes of *A. galanga* (L.) Wild collected from Vietnam, with the main volatile compositions including limonene (8.32%), *α*-terpinene (6.72%), camphene (5.98%), *α*-pinene (2.14%), *β*-pinene (1.93%) and *α*-farnesene (3.89%), was 47.15% (Van Loi and Uyen 2016), while the EO from *A. galanga's* rhizome collected in Thailand, in which 1,8-cineole (46.22%), 4-allylphenyl acetate (9.38%), *β*-bisabolene (6.04%), *β*-pinene (5.21%), *α*-pinene (4.34%) were the main compositions, showed higher DPPH scavenging activity, with the IC_{50} value of 0.55 mg/mL (Tachakittirungrod and Chowwanapoonpohn 2007). In another study, the DPPH scavenging activity of *Viola hamiltoniana*'s EOs was 15.72% at a concentration of 100 mg/mL and was not raised at a higher concentration (Zhang and Lai 2017). Taken all together, PS-EO and RH-EO showed weak scavenging activities against DPPH and ABTS free radicals in comparison with the positive control, Ascorbic acid. PS-EO showed stronger activities compared with RH-EO.

As for metal-reducing powers, PS-EO and RH-EO showed concentration-dependent reducing powers for ferric (FRAP) (Figure S6) and cupric ions (CUPRAC) (Figure S7). The EC_{50} values of PS-EO and RH-EO were 3.50 and 23.00 mg/mL for ferric reducing powers and 5.30 and 19.00 mg/mL for cupric reduction, respectively (Table S4). PS-EO and RH-EO again showed weaker activities in comparison with the positive control, as the EC_{50} values of Ascorbic acid were 0.07–0.08 mg/mL. The degree of this difference was smaller than that of scavenging activities. It was worth noting that the FRAP assay was conducted at a low pH, which facilitates the transition of electrons from antioxidant agents to metal ions, while the CUPRAC reaction occurred at a neutral pH. The results, however, indicated that the reducing powers of PS-EO and RH-EO were not significantly affected by pH conditions ($p = 0.39 > 0.05$).

The results also showed that the antioxidant activities of PS-EO were higher than those of RH-EO. This could be explained based on their differences in chemical composition, especially in two major groups: monoterpenes and total phenolic compounds. For monoterpenes, this group was well studied not only for their antimicrobial but also for their antioxidant activities (Bouchekrit et al. 2016; Loizzo et al. 2016). As the proportions of monoterpenes in PS-EO were approximately twice as high as in RH-EO (Table S1), this could result in the higher activities of PS-EO regarding scavenging and reducing powers than those of RH-EO. The difference in monoterpene constitutions may also matter. The higher levels of *β*-pinene, *α*-pinene and citral in PS-EO than in RH-EO could contribute to the gap in their antioxidant activities. A previous study revealed that higher doses of *α*-pinene increased DPPH and FRAP activities (Bouzenna et al. 2017). Limonene, *γ*-terpinene and *β*-pinene were also considered the main compounds of EOs that exhibited good scavenging activities against DPPH and FRAP (Loizzo et al. 2016). In contrast to previous studies, considering 1,8-cineole main compound in the EO from *A. officinarum* from China (Zhang et al. 2010) or terpinen-4-ol the highly active antioxidant compound in the EO from *A. coriandriodora* D. Fang (Dong et al., 2020), the higher amounts of 1,8-cineole and terpinene-4-ol in RH-EO did not result in its higher antioxidant activities compared with PS-EO. This could be explained that the antioxidant activities of the EOs are the synergetic effects of all compositions and are not attributed to one or some active compounds.

In addition to monoterpenes, phenolic compounds are the main constituents that directly affect the antioxidant properties of essential oils. The lower content of total phenols in RH-EO could be a reason for its weaker antioxidant activities than PS-EO. A strong positive correlation was shown between TPC and DPPH and ferric-reducing power in EOs from *A. zerumbet* and *A. conchigera*, collected from the Northeastern region of Thailand (Chumroenphat et al. 2019).

3. Conclusions

In conclusion, this study first revealed the chemical composition and antioxidant activities of essential oil extracted from the rhizomes and pseudo-stem of *A. nelumboides*'s. The main volatile compounds in PS-EO were 1,8-cineole, (*E*)-citral, *β*-pinene, *α*-pinene, limonene and geranyl acetate, while 1,8-cineole, linalool, (*E*)-citral, (*Z*)-citral, *α*-pinene, limonene and *β*-pinene predominated in RH-EO. PS-EO contained higher levels of polyphenols and monoterpenes than RH-EO. Both the EOs exhibited scavenging and reducing powers, but the antioxidant activities of PS-EO were much more pronounced than those of RH-EO, indicating the strong correlation of the content of monoterpenes and polyphenols to antioxidant activities. Other phytochemicals of *A. nelumboides* and their bioactive activities should be further studied to provide more comprehensive information on phytochemical profiles of this new species and open opportunities for other practical applications.

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