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The Presence of Monoterpenes in *Rhynchanthus longiflorus* Hook.f. Confirms the Value of Its Use in Akha Folk Medicine

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Abstract:

Background: *Rhynchanthus longiflorus* Hook.f. is an endangered species and is part of the ginger family. A decoction of its rhizomes is mentioned in Akha folk medicine for the treatment of convulsions.

Objective: With no prior studies performed, it was reasonable to analyze the chemical components of the rhizomes responsible for the odor, taste and perhaps medicinal claims for the plant.

Methods: Rhizomes of *Rhynchanthus longiflorus* Hook.f. were bought from local markets in Chiang Rai, Thailand. They were subjected to hydrodistillation to obtain the essential oil. Analysis of the oil was done by GC-MS.

Results: Fifteen monoterpenes were identified from the essential oil. The most abundant compounds were 1, 8-Cineol (70.705 %), beta-Pinene (7.008%), alpha-Pinene (4.763%) and Sabinene (3.484%) which comprised 85.96 % of the oil. These monoterpenes have been reported to reduce mortality and attenuate seizures in animal models

Conclusion: The essential oil from *Rhynchanthus longiflorus* Hook.f. rhizomes contains monoterpene compounds whose anticonvulsant effects could be the basis for the use of this plant in Akha folk medicine.

Key words: Akha, *Rhynchanthus longiflorus* Hook.f., Monoterpene

Introduction

There are six major tribes or minorities living in the remote hills of northern Thailand. They are the Akha, Mhong, Karen, Lahu, Lisu and Mien. Folk medicine, in which medicinal plants play a major role, has been popularly used by these tribes in the maintenance of health as well as in the prevention, diagnosis, and treatment of physical and mental illness[1,2]. Among the medicinal plants used by these tribal people, *Rhynchanthus longiflorus* Hook.f.,

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an endangered species in the ginger family, is occasionally mentioned. The plant is an epiphytic, perennial rhizomatous herb which can be easily characterized by a profound inflorescence that looks like a bird[3]. Only in some Akha villages, the evidence for its medicinal use was narrated by folk healers, who said that the decoction of the plant's rhizomes could be used to treat convulsions[4].

In May 2015, we found rhizomes of a plant resembling *Rhynchanthus longiflorus* Hook.f. (Figure 1) being sold in the walking street market of Mae Chan district, Chiang Rai province, Thailand. The vendor referred to these rhizomes by an Akha word that means "cliff ginger" and told us that he bought them from the Kentung market in Myanmar. The rhizomes were 4.5–5 x 2.5–3 cm, ovate, faintly aromatic with membranous scale leaves, arranged in a single linear row. There was no inflorescence or other recognizable plant parts, essential for authentication. Therefore, to ascertain that these rhizomes belonged to *Rhynchanthus longiflorus* Hook.f., we cultivated them until their inflorescences appeared.

Convulsion is a neurological disorder which commonly controlled by the anticonvulsants. However, some patients have been experienced uncontrolled seizures and serious side effects due to the drugs[5]. Therefore, finding of new candidate compound with high efficacy and good safety profile would be a valuable strategy for the convulsion control. The rhizome of *Rhynchanthus longiflorus* Hook.f has been previously mentioned for its traditionally use as anticonvulsants. However, there is a lack of robust evidence for the medicinal claims. To fill this gap, we therefore analyzed the chemical components of the rhizomes responsible for the odor, taste, and medicinal claims of the plant.

Materials and Methods

Plant material

Approximately 10 kg. of the rhizomes were bought from the walking street market of Mae Chan district, Chiang Rai province, Thailand in May, 2015. Some of the rhizomes were cultivated in plastic baskets, containing coconut husk chips and pumice, until the inflorescence emerged from the leafy shoot six months later. The whole plant including its inflorescent was assigned as CR-019 and sent to the Queen Sirikit Botanical garden for identification.

Preparation of the essential oil

Coarsely chopped fresh rhizomes (6567.26 g) were hydrodistilled in a Clevenger apparatus After extraction, the organic layer (essential oil) was separated from water by decantation and then desiccated by slow percolation over anhydrous sodium sulphate (Na_2SO_4) in a simple filtration system. The essential oil was stored in amber color vials in cool and dark conditions awaiting further analysis.

Analysis of chemical components by GC-MS

Phytochemical analysis of the essential oil was accomplished using GC-MS analysis. The system comprised a gas chromatograph (GC 7890 Agilent Technologies Co., Ltd., USA) and a mass selective detector (Agilent HP 5973) using a DB-5MS column (30 m x 0.25 mm i.d., 0.25 μm film thickness) with electron ionization (70 eV) and an ion source operated at a temperature of 230°C. The GC oven temperature was set as follows: commence at 50°C and held for 5 min, then slowly raised (1°C/min) to 200°C, and then to final temperature, 250°C (5°C/min) which was maintained for 10 min. The temperature of the injector was held at 250°C; the flow rate of the helium (carrier gas) was maintained at

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1.5 mL/min; 1:25 split ratio. About 1 µl of the essential oil was injected into the GC-MS using a micro syringe and the scanning was done for 45 min. As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal whenever a compound was detected. The greater the concentration in the sample, bigger was the signal obtained, which was then processed by computer. The time from when the injection was made (Initial time) to when elution occurred is referred to as the retention time (RT). The chemical constituents of the extract were identified by comparing the relative retention times and mass spectra with the data in the NIST05a.L

Results

Plant material

Approximately six months after cultivation of the rhizomes, a beautiful decorative inflorescence emerged from each leafy shoot. The leaves were sessile or shortly petiolate with oblong-lanceolate or elliptic-oblong leaf blades. The inflorescence, 9–10 cm, was an unbranched spike with a short peduncle and a bright red axis slightly curved upwards with 5–6 flowers, all directed upwards as shown in Figure 1. The plant sample (CR-019) was identified by Dr. Jarun Maknoi, Queen Sirikit Botanical garden, Thailand as *Rhynchanthus longiflorus* Hook.f. and the herbarium number, QBG. No. 85757, was assigned to the specimen.



Figure 1 Rhizomes, leafy shoot and inflorescence of *Rhynchanthus longiflorus* Hook.f

Preparation of the essential oil

The chopped rhizomes had an aromatic camphoraceous odor with a pungent, spicy, and cooling taste. From 6567.26 g of the fresh rhizome, 20.0 ml of the essential was obtained. The essential oil yield was thus 0.304 % (w/w).

Analysis of chemical components by GC-MS

Sixteen peaks were distinguished by their retention times. One compound at the retention time of 12.506 min could not be identified. However, the content of this unknown compound was only 0.581% and so could be assumed as negligible. The most abundant monoterpenes from the 15 identified compounds found in the essential oil were 1,8-Cineol (70.705 %), beta-Pinene (7.008%), alpha-Pinene (4.763%) and Sabinene (3.484%) which comprised 85.96 % of the oil (Table 1).

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Table 1 Essential oil composition (%) of *Rhynchanthus longifolius* rhisomes

Compounds	Retention time (min)	Content (%)
alpha-Pinene	7.762	4.763
Sabinene	8.740	3.484
beta-Pinene	8.804	7.008
beta-Myrcene	9.142	0.884
3-Carene	9.547	0.538
1, 8-Cineol	10.077	70.705
beta-Ocimene	10.337	0.644
gamma-Terpinene	10.548	0.602
beta-Linalool	11.318	2.515
Unknown	12.506	0.581
4-Terpineol	12.682	1.819
alpha-Terpineol	12.899	2.630
2-Carene	15.226	0.967
trans-Methyl isoeugenol	15.937	1.485
beta-Patchoulene	18.885	0.356
beta-Eudesmol	19.110	1.019

Discussion

The genus Rhynchanthus is represented by six species distributed in Myanmar, India and South China[6]. The Akha tribe, who are aware of the medicinal use of *Rhynchanthus longiflorus* Hook.f. originated in South China but migrated into Myanmar and Thailand around the middle of the nineteenth century[7]. In the present study, we analyzed the chemical components of *Rhynchanthus longiflorus* Hook.f responsible for the odor, taste, and medicinal claims. The Essential oils in which mono-terpenes are the major components of the plant. They have been shown to exert anticonvulsant activity. In 2009, Oliveira et al. reported that the essential oil of Ocimum basilicum increased the latency for development of convulsions in pentylenetetrazol-induced convulsions in mice. The principal components of the oil were 1.8-cineole, linalool, and geraniol, which comprised 92.9% of the oil[8]. Again in 2016, Masoumi-Ardakani et al. demonstrated the anticonvulsant activity of the essential oil of Elettaria cardamomum, an aromatic spice (cardamom) native to the humid Asian areas, in pentylentetrazole and maximal electroshock models in mice. The major compounds in the essential oil were 1,8-cineole (45.6 %), α-terpinyl acetate (33.7 %), sabinene (3.8 %), 4-terpinen-4-ol (2.4 %), and myrcene (2.2 %), respectively[9].

Monoterpenes are characterized by having two isoprene units, each with five carbons (C5) in the structure[10]. One of the key properties of monoterpenes, in the context of epilepsy, is their ability to cross the blood-brain barrier (BBB). Drug candidates likely to cross the BBB are usually of small

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size (<400 Da) and high lipid solubility[11]. Virtually all the monoterpenes found in the essential oil of *Rhynchanthus longiflorus* Hook.f. meet these criteria. Monoterpenes also show anti-inflammatory[12] and antioxidant activities[13,14]. These proprieties make them interesting molecules for protection against neuro-inflammation and redox disequilibrium, which have been observed in the neurological disorders occurring in epilepsy[15-17].

Of all the monoterpenes found in this essential oil, 1,8-Cineole, alpha-Pinene, beta-Pinene, Alpha-Terpineol, beta-Linalool and beta-Myrcene, have been shown to reduce mortality and attenuate seizures in animal models[18-23]. We found no reports of anticonvulsant effect of Sabinene but the oil from the dried seed kernel of nutmeg (*Myristica fragrans* Houtt), in which Sabinene was the major active constituent, was demonstrated to exhibit anticonvulsive action[24]. Therefore, it is reasonable to postulate that the presence of these monoterpenes in the essential oil of *Rhynchanthus longiflorus* Hook.f. was responsible for the anticonvulsant effect of this medicinal plant.

Conclusion

In conclusion, the essential oil from *Rhynchanthus longiflorus* Hook.f. rhizomes contains monoterpene compounds whose anticonvulsant effect could be the basis for the use of this plant in Akha folk medicine.

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Conflicts of Interest

The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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