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Abstract

Keywords: separated by comma, keyword1, keyword2

Running title:

Introduction

The plant *Zygostelma benthamii* Baill **(Figure 1)** previously a member of Asclepiadaceae but now belongs to Apocynaceae known in Thai as “Op Choei Thao”, the root has been used in folk medicine as a carminative, a heart tonic, an antispasmodic, and to treat vertigo. The stems are used to treat headache and lumbo-sacral pain. It is a slender, laticiferous, twining, sometimes prostrate or semi-erect shrub. Roots are woody and aromatic. The stem is numerous, slender, terete, thickened at the nodes. Often with milky latex, leaves are sparse, opposite and highly variable in shape and length; linear-lanceolate to elliptic-oblong, 2.5-10 cm long and 0.7-3.8 cm broad. Dorsal parts are often variegated in white with silvery white and pubescent beneath. Flowers are small, opposite, crowded in subsessile axillary cymes and greenish outside and purplish inside. Fruit is 10-15 cm long cylindrical, glabrous follicle with several black flattened seeds with long white silky hairs [3-7].

Figure 1. Figure caption.

Materials and Methods (allows subheadings, no further headings. Make it reproducible, but simple)

Plant materials

Zygostelma benthamii Baill roots were collected from Phetchaburi province, Thailand in May 2004, A herbarium specimen was labeled as NP 2004-01 and deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University. Authentication was achieved through comparison with herbarium specimens in the Forest Herbarium National Park, Wildlife and Plant Conservation Department Ministry of Natural Resources and Environment, Thailand.

Extraction and isolation

The fresh roots of *Zygostelma benthamii* root (1 kg) were reduced to fine particles in a blender and macerated with MeOH (3x1000 mL) for one week at room temperature. The extracts were combined and filtered. The filtrate was concentrated under reduced pressure yielding white precipitate (20 g) which was filtered off. The filtrate was evaporated to dryness giving a brownish residue (50.0 g) with characteristic odor. The crude MeOH extract (50.0 g) was suspended in 300 mL water and partitioned with n-hexane, EtOAc, and n-BuOH respectively. The organic layers were evaporated under reduced pressure to provide *n*-hexane solubles (2.0 g), EtOAc solubles (3.3 g), and n-BuOH solubles (6.0 g). The aqueous layer was evaporated to dryness on a water bath to give a water-soluble fraction (30 g).

Results & Discussion (can be separated)

Table 1. Parameters of method validation for α-amyrin acetate and β-lupeol acetate quantification by TLC densitometry.

Parameter	α-amyrin acetate	β-lupeol acetate

Linearity range (µg/spot)	1.0-8.0	1.0-8.0
Linearity (r^2, Correlation coefficient)	0.996	0.997
Precision (%RSD)		
Repeatability of measurement (n=5)	0.60	0.96
Intra-day precision	0.62	1.83
Inter-day precision		
Accuracy (% recovery)		
Level 1 (4 µg/spot)	98.45	98.24
Level 2 (6 µg/spot)	97.06	97.85
Limit of detection (µg/spot)	0.01	0.02
Limit of quantification (µg/spot)	0.05	0.06

Conclusion (can be removed, limited to one or two paragraphs)

Zygostelma benthamii Baill is known in Thai as “Op Choei Thao". The botanical characteristics of this plant resembles *Hemidesmus indicus* (Linn.) R.Br., an Indian medicinal plant, which belongs to the same family, Apocynaceae. The most obvious characteristics of both plants are the dark green leaves variegated with white midribs and large obvious veins.

Their traditional indications shared some similarities. The roots of *Z. benthamii* have been used traditionally as carminative, heart tonic, antispasmodic, and antivertigo. These uses suggested anti-inflammatory, antioxidant, and antimicrobial properties. Previous study also found that the root extract also possessed anti-tyrosinase activity.

Declarations

Availability of data and materials

Competing interests

Funding

Authors' contributions

Supporting information

The Supporting Information is available free of charge at the Journal Website.

Acknowledgement

References (book chapter and research article in English only, no webpage, patent, unpublished data)

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