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Chemodiversity of *Clausena excavata* (Rutaceae) and related species: Coumarins and carbazoles

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ABSTRACT

Methanolic crude extracts obtained from leaves, stem bark and root bark of 57 accessions of *Clausena excavata*, *C. harmandiana*, of both varieties of *C. wallichii,* as well as of three unidentified *Clausena* samples, were analysed for the presence of coumarins and carbazoles. All accessions were collected from different locations all over Thailand. HPLC coupled with UV-DAD was applied for phytochemical profiling. Structures of known pyranocoumarins **1–4** and of coumarins **5–8** bearing an *O*-geranyl moiety, as well as of carbazoles **9–12** were confirmed by NMR and MS spectroscopy, respectively. Observed chemical diversification in the studied samples is discussed in relation to geographic distribution and with respect to organ-specific accumulation. Chemotaxonomic significance is briefly addressed.

1. Introduction

Species of the small palaeotropical genus *Clausena* Burm.f. (Aurantioideae, Rutaceae) are occurring in several forest types throughout Africa, South and South-East Asia. More specific, *Clausena* species occur between Southern China and NE-Australia and Western Africa until New Guinea. According to Molino (1994) *Clausena* comprises of about 15 species and six varieties, all of them are trees or shrubs, of which five species are distributed in Thailand. Several species produce edible fruits such as *C. lansium* (Lour.) Skeels and some other species are widely used in local folk medicine and for other purposes throughout the whole distribution area. For example, locals in China and Thailand use leaf extracts of *C. excavata* Burm.f. and *C. lansium* to cure cold, abdominal pain, malaria, dysentery and many other diseases (Adebajo et al., 2009; Nakamura et al., 2009; Arbab et al., 2011). The species *C. harmandiana* (Pierre) Guillaumin is used as a health promoting plant in Thailand (Yenjai et al., 2000). Insecticidal properties of *C. anisata* (Willd.) Hook.f. ex Benth. extracts are responsible for its use in insect pest control in Africa by local people (Mukandiwa et al., 2016). Members of this genus are characterized by the accumulation of coumarins, of 3-methyl carbazoles and their oxidized derivatives, and of tryptamine-derived amides (Chakraborty et al., 1995; Herdits-Riemer, 2002; Riemer et al., 1997; Samuel et al., 2001). Especially coumarins and carbazoles possess pronounced bioactivities (eg. Huang et al., 1997; Du et al., 2015; Ito et al., 2009; Xia et al., 2015; Greger, 2017; Ma et al., 2017). Furthermore, tryptamine-derived amides were reported from *C. lansium* (Riemer et al., 1997; Wang et al., 2013; Xia et al., 2014). Carbazoles were described from roots and twigs from *C. wallichii* Oliv. (Maneerat et al., 2012, 2013a), while *C. harmandiana* yielded bioactive carbazoles and coumarins (Maneerat et al., 2013b; Songsiang et al., 2012; Wangboonskul et al., 2015). Recently, carbazole and quinoline alkaloids were reported from leaves and stems of *C. dunniana* H.Lév. (syn. *C. anisata*) (Cao et al., 2018). Leaves of *Clausena* species also contain a broad spectrum of bioactive volatile compounds (Diep et al., 2009; Nath et al., 1996; Poonkodi et al., 2017) which might also be responsible for the often distinct smell.

Clausena excavata, which is focus of this study, is known for accumulation of carbazoles and coumarins, together with terpenoids (eg. Thuy et al., 1999; Wu et al., 1993). However, little attention was paid so far on chemical diversification of geographically distinct *C. excavata* populations and closely related species. Similarly, organ-specific accumulation within species and individuals was addressed rarely in those studies cited above. For this purpose, we studied 57 accessions belonging to the three species, *C. excavata* Burm.f. (23 individuals), *C. harmandiana* (five), *C. wallichii* (26), including three unidentified *Clausena* samples. The plant material was collected within the last three

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Table 1
Sampling data of the examined plant material.

Species	Origin	Floristic region	Herbarium voucher
C. excavata	S. Nakhon, KU Campus	NE	2015-NW02
	N. Ratchasima, Sakaerat	E	2015-NW09
	N. Ratchasima, Sakaerat	E	2015-NW12
	N. Ratchasima, Sakaerat	E	2015-NW13
	Phangnga, Hat Ao Khoei	Pen	2015-NW18[a]
	S. Nakhon, Meung Distr.	NE	2016-NW32
	S. Nakhon, Meung Distr.	NE	2016-NW34
	S. Nakhon, Meung Distr.	NE	2016-NW35
	S. Nakhon, KU Campus	NE	2016-NW39
	S. Nakhon, KU Campus	NE	2016-NW41
	S. Nakhon, KU Campus	NE	2016-NW42
	Trat, Agroforestry Res. & Training Station	SE	2016-NW54
C. cf.*excavata*	S. Nakhon, KU Campus	NE	2015-NW05
	N. Ratchasima, Sakaerat	E	2015-NW10
	N. Ratchasima, Sakaerat	E	2015-NW11
	Chumphon, Pathio Distr.	Pen	2015-NW15
	Chumphon, Pathio Distr	Pen	2015-NW16
	S. Nakhon, Meung Distr.	NE	2016-NW20
	S. Nakhon, Meung Distr.	NE	2016-NW28
	Trat, Agroforestry Res. & Training Station	SE	2016-NW51
	Trat, Agroforestry Res. & Training Station	SE	2016-NW53
	Trang, Wang Wiset Distr.	Pen	2016-NW63
	Trat, Hat Ploy Dang	SE	2017-NW78
C. harmandiana	Prachin Buri, Si Mahosot Distr.	SE	2016-NW70
	Chiang Mai, Mae Rim Distr.	N	2017-NW71
C. cf. *harmandiana*	Ratchaburi, Photharam Distr.	SW	2016-NW66
	Ratchaburi, Photharam Distr.	SW	2016-NW67
	Ratchaburi, Photharam Distr.	SW	2016-NW68
C. wallichii var. *guillauminii*	S. Nakhon, Meung Distr.	NE	2016-NW21
	S. Nakhon, Meung Distr.	NE	2016-NW22
	S. Nakhon, Meung Distr.	NE	2016-NW26
	S. Nakhon, Meung Distr.	NE	2016-NW29
	S. Nakhon, Meung Distr.	NE	2016-NW30
	S. Nakhon, Meung Distr.	NE	2016-NW33
	S. Nakhon, Meung Distr.	NE	2016-NW36[a]
	S. Nakhon, Meung Distr.	NE	2016-NW37
	S. Nakhon, Meung Distr.	NE	2016-NW38
	S. Nakhon, KU Campus	NE	2016-NW40
	S. Nakhon, KU Campus	NE	2016-NW43
	S. Nakhon, KU Campus	NE	2016-NW44
	S. Nakhon, KU Campus	NE	2016-NW45
	S. Nakhon, KU Campus	NE	2016-NW46
	S. Nakhon, KU Campus	NE	2016-NW47
	S. Nakhon, KU Campus	NE	2016-NW50
C. cf. *wallichii* var. *guillauminii*	S. Nakhon, Meung Distr.	NE	2016-NW23
	S. Nakhon, Meung Distr.	NE	2016-NW24
	S. Nakhon, Meung Distr.	NE	2016-NW25
	S. Nakhon, Meung Distr.	NE	2016-NW27
	S. Nakhon, Meung Distr.	NE	2016-NW31
	S. Nakhon, KU Campus	NE	2016-NW48
	S. Nakhon, KU Campus	NE	2016-NW49
C. cf. *wallichii* var. *wallichii*	Loei, Na Haeo Distr.	NE	2018-NW82
	Loei, Na Haeo Distr.	NE	2018-NW83
	Loei, Na Haeo Distr.	NE	2018-NW84
Clausena sp.	Uthai Thani, Lan Sak Distr.	SW	2017-WA198
Clausena sp.	Chiang Mai, Mae Rim Distr.	N	2017-NW72
Clausena sp.	Chiang Mai, Mae Rim Distr.	N	2017-NW73

[a] Used for isolation; N. Ratchasima = Nakhon Ratchasima, S. Nakhon = Sakhon Nakhon. Floristic regions: Eastern (E), Northern (N), North-Eastern (NE), South-Eastern (SE), South-Western (SW), Peninsular (Pen). The samples were collected by N. Wongthet and W. Aiyakool.

years in various floristic regions of Thailand (Pooma and Suddee, 2014) (Table 1). Diversification of the observed chemical profiles is discussed in relation to geographic distribution, and chemotaxonomic aspects are briefly addressed.

2. Material and methods

2.1. General experimental procedures

TLC—Thin Layer Chromatography was performed on silica gel 60 F_{254} plates (Merck) using a solvent system of hexane and ethyl acetate (EtOAc) (6:4) sprayed with anisaldehyde. For prep. TLC plates of 0.5 mm thickness were used.

HPLC—HPLC analyses were performed on Agilent 1100 Series equipped with an UV-DAD detector, Hypersil BDS-C18 column (250 × 4.6 mm, 5 µm particle size), eluted with MeOH in aq. buffer (15 mM ortho-H_3PO_4 and 1.5 mM Bu_4NOH) with a flow rate of 1.0 mL/min, injection volume of 10 µL and linear gradient starting from 60% MeOH to 90% at 17 min to 100% at 20 min kept for 8 min. The detection signal was set at 230 nm.

Column chromatography (CC)— silica gel 60 with 0.2–0.5 mm or 40–63 µm particle size was used. The eluents were mixtures of petrol