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Optimization of extraction techniques and quantification of swertiamarin and mangiferin by using RP-UFLC method from eleven *Swertia* species

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ABSTRACT

Present work investigated an efficient method for extraction of swertiamarin and mangiferin from different species of genus *Swertia*. Various extraction methods like static extraction (SE), continuous shaking extraction (CSE), and ultrasonic extraction (USE) were evaluated for increasing recovery percentage of swertiamarin and mangiferin. The quantification was done using reversed phase-ultra flow liquid chromatographic (RP-UFLC) method at 238 nm (swertiamarin) and 257 nm (mangiferin) wavelength. The results revealed that the percentage extraction of swertiamarin and mangiferin from different species of *Swertia* by SE was more proficient. Three time intervals were optimized by SE and it was observed that the 24 h extraction gave the maximum recovery in *S. chirayita* of swertiamarin (256.98 mg/g) and mangiferin (155.76 ± 7.78 mg/g). *S. minor* remarkably best match for *S. chirayita* as per phytochemical fingerprint using swertiamarin and mangiferin is concerned, suggesting an alternative for chirayita.

1. Introduction

Family Gentianaceae is represented by 84 genera and ~970 species in world (Judd et al., 1999). Members of this family are widely distributed throughout the world, but are most diverse in temperate, subtropical and montane tropics. About 170 known *Swertia* species are mainly native to temperate regions of the Northern hemisphere and about 40 species, mainly found in the temperate Himalayan region ranging from Kashmir to Bhutan, Khasia and Western Ghats hills (Anonymous, 1982; Scartezzini and Speroni, 2000; Brahmachari et al., 2004). Due to immense national and international trade value and scarcity of chirayita, other *Swertia* species are reported to be adulterant to *S. chirayita* (Joshi and Dhawan, 2005; Kumar and Staden, 2015).

Swertia species are reported for presence of compound groups such as glycosides, seco-irridoids and xanthones which are known to have therapeutic effects and pharmacological activities (Brahmachari et al., 2004; Phoboo et al., 2010; Negi et al., 2011; Kshirsagar et al., 2015). Specifically, swertiamarin (seco-iridoid glycoside) has showed inhibition of human DNA ligase I (Tan et al., 1996), antiedematogenic, antioxidant, hepatoprotective (Jaishree and Badami, 2009, 2010), antinociceptive (Jaishree et al., 2009), antihyperlipidimic (Vaidya et al., 2009), gastroprotective, antiulcerogenic, antichlonergic and CNS depressant (Bhattacharya et al., 1976; Yamahara et al., 1991; Soni and Gupta, 2009). Swertiamarin content evaluated in different *Swertia* species by using different techniques has been depicted in Table 1.

On other hand, naturally occurring xanthones are a group of organic compounds emerged out as an important pharmacological and biological activities. Xanthones especially mangiferin was first xanthone to be investigated pharmacologically and showed broad spectrum of biological activities and is widely distributed in higher plants such as Anacardaceae and Gentianaceae families. Mangiferin exhibits diverse pharmacological activities separately or collectively scrutinized by Kshirsagar et al. (2016). Mangiferin content was evaluated in different *Swertia* species by using wide range of techniques has been listed by Kshirsagar et al. (2016) and also depicted in Table 2.

Abbreviations: CSE, Continuous shaking extraction; SE, Static extraction; USE, Ultrasonic extraction; LOD, Limit of detection; LOQ, Limit of quantification; RP-UFLC, Reverse phase-ultra flow liquid chromatography; RSD, Relative standard deviation; SAN, *Swertia angustifolia* (PRK-21); SAP, *Swertia angustifolia* var. *pulchella* (PRK-14); SBI, *S. bimaculata* (PRK-17); SCH, *S. chirayita* (PRK-20); SCO, *S. corymbosa* (PRK-13); SDE, *S. densifolia* (PRK-7); SDI, *S. dilatata* (PRK-19); SLA, *S. lawii* (PRK-11); SMI, *S. minor* (PRK-5); SNE, *S. nervosa* (PRK-16); SPA, *S. paniculata* (PRK-18).

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Table 1
Content of swertiamarin (mg/g) reported in different *Swertia* species.

Sr. no.	Species	Content (mg/g)	Method	Extraction	References
1	*S. mussotii*	26.9	HPCE	Sample was soaked with 10 ml methanol for 24 h in dark, add running buffer and extracted for 30 min in an ultrasonic bath	Cao et al. (2005)
2	*S. cordata*	55.9	HPLC	Samples extracted with ethanol–water, 50:50 (v/v), with sonication (2 × 30 min, 45 °C)	Bhandari et al. (2006)
3	*S. chirata/chirayita*	4.4	HPLC		
4	*S. densiflora/densifolia*	2.94	HPTLC	0.5 g sample extracted with 25 ml of methanol. The residue reconstituted with 25 ml of chloroform and vortexed. Filtrate evaporated and residue was again reconstituted in 10 ml of methanol	Shailajan and Abhishek (2009)
5	*S. mileensis*	NM	NM	NM	Zhou et al. (2006)
6	*S. chirayita*	1.28	HPLC	1 g sample was mixed with 100 ml of either water or 12% ethanol and left mixture at room temperature (25 °C) for 12 h	Phoboo et al. (2010)
7	*S. franchetiana*	0.5–3	HPLC	Methanol (70%) was used to extract	Yang et al. (2004)
8	*S. mussotii*	1.52–3.63	HPLC	70% methanol used for extraction	Yang et al. (2005)
9	*S. chirayita*	–	LC–ESI/MS	Materials were homogenized with methanol. The resultant extracts were centrifuged for 10 min at 3000 rpm and the supernatant was used for analysis	Kumar and Chandra (2015)
10	*S. chirata*	–	LC–ESI/MS	–	Suryawanshi et al. (2006)
11	*S. mileensis*, *S. patens*, *S. yunnanensis and S. delavayi*	34.47–118.05	HPLC	–	Li et al. (2013)
12	*S. franchetiana*	1.84	HPLC	1.529 g sample was extracted in10 ml methanol for 1 h In an ultrasound bath	Li et al. (2007)
13	*S. japonica*	38.51–40.53	HPLC-DAD-MS	1 g sample extracted twice over a period of 1 h each with 15 ml of methanol, extracts evaporated and residue was dissolved in 10 ml of methanol	Wang et al. (2008)
14	*S. pseudochinensis*	10.83	HPLC-DAD-MS		
15	*S. delavayi*	29.36	HPLC-DAD-MS		
16	*S. decora*	37.23	HPLC-DAD-MS		
17	*S. binchuangensis*	6.37	HPLC-DAD-MS		
18	*S. punicea*	1.36	HPLC-DAD-MS		
19	*Swertia herb (Market)*	64.28–84.25	CE	10 mg of sample extracted with 40 ml of water by shaking for 15 min	Takei et al. (2001)
20	*S. chirayita*	16.7–84.7	HPTLC	Samples were defatted with 20 ml n-Hexane for 48 h. The residue was extracted with 20 ml methanol for 72 h.	Samaddar et al. (2013)
21	*S. bimaculata*	4.8–58.0	HPTLC		
22	*S. nervosa*	1.5	HPTLC		
23	*S. dilatata*	8.6	HPTLC		
24	*S. paniculata*	8.8	HPTLC		
25	*S. chirayita*	0.13	TLC GelQuant.NET	Samples were extracted with 100% methanol (10% w/v) and ultrasonication for 2 h	Khanal et al. (2015)
26	*S. angustifolia*	0.15	TLC GelQuant.NET		
27	*S. paniculata*	0.08	TLC GelQuant.NET		
28	*S. racemosa*	0.039	TLC GelQuant.NET		
29	*S. nervosa*	0.04	TLC GelQuant.NET		
30	*S. ciliata*	0.01	TLC GelQuant.NET		
31	*S. dilatata*	0.16	TLC GelQuant.NET		
32	*S. minor*	2.5–145.3	HPLC	Extracts were prepared in methanol and extracted for 24 h at room temperature	Kshirsagar et al. (2015)
33	*S. densifolia*	20.2–93.74	HPLC		
34	*S.lawii*	22.23–127.44	HPLC		
35	*S. corymbosa*	119.33	HPLC		
36	*S. angustifolia var. pulchella*	83.21	HPLC		
37	*S. chirayita*	130.53	HPLC		
38	*S. punicea*	6.72–128.94	HPLC	Samples were extracted twice with 10 ml methanol in an ultrasonic bath at 45 °C for 30 min. Extracts evaporated to dryness, and the residue was dissolved with methanol	Tian et al. (2008)
39	*S. kouichensis*	11.23	HPLC		
40	*S. bifolia*	0.94	HPLC		
41	*S. cincta*	Trace	HPLC		
42	*S. macrosperma*	Trace	HPLC		
43	*S. diluta*	16.43	HPLC		
44	*S. erythrosticta*	Trace	HPLC		

The detailed literature survey, suggested that the studies carried out to optimize yield of compound/s has been restricted to one or two plants (Fulzele and Satdive, 2005; Pai et al., 2011). There are number of methods identified for extraction of plant based metabolites *viz*. camptothencin (Fulzele and Satdive, 2005), gingerol (Pawar et al., 2010), swertiamarin (Table 1), and mangiferin (Table 2). Tables 1 and 2 depicts previous studies done on various *Swertia* species with reference to swertiamarin and mangiferin content. The tables also provide details on content, extraction technique and method for detection used in quantifying these contents. It can be inferred from this table that the variation in content is certainly due to use of various extraction and recovery methods. Extraction and product recovery are supposed to be the most imperative steps in evaluation of target molecules. Most of the extraction processes are time consuming, laborious, involves lengthy operation techniques. Two major parameters affecting content yield of analytes, includes method of extraction and time required for it. The order of magnitude in the yield may vary with respect to even slight change in these parameters. Accordingly, it is essential to regulate these factors and in turn to understand the correct method to attain greater accuracy in the results.

Thus, the present study deals with identifying suitable extraction method and time of exposure to get optimum yield of swertiamarin and mangiferin from11 species of *Swertia* from India. To the best of authors knowledge, there are no such reports on the targeted compounds in *Swertia* species.

2. Materials and methods

2.1. Collection of plant materials, extract and standards preparation

Plant material of eleven *Swertia* species were obtained from different localities of Western Ghats and Eastern Himalayan region. Specimen