



Original Article

 Metabolic profile and β -glucuronidase inhibitory property of three species of *Swertia*


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ABSTRACT

β -Glucuronidase inhibitors are suggested as potential hepatoprotective agents. *Swertia chirayita* (Roxb.) Buch.-Ham. ex C.B. Clarke, Gentianaceae, is known for its hepatoprotective and anti-hepatotoxic activity in Ayurvedic system of medicine for ages. This plant is substituted by other species like *S. decussata* Nimmo ex C.B. Clarke and *S. bimaculata* (Siebold & Zucc.) Hook. f. & Thomson ex C.B. Clarke. The aim of the study was to compare metabolite profile and β -glucuronidase inhibitory activity of these three important species of *Swertia* and to identify the active constituents. *S. chirayita* (IC₅₀ 210.97 μ g/ml) and *S. decussata* (IC₅₀ 269.7 μ g/ml) showed β -glucuronidase inhibitory activity significantly higher than that of silymarin, the known inhibitor of the enzyme. The activity of *S. bimaculata* was low. The metabolites present in the three species were analyzed by HPLC and GC-MS based metabolomics approach. Five amino acids, twenty one organic acids, one inorganic acid, eight fatty acids, twenty one phenols including xanthones, eight sugars, seven sugar alcohols, five terpenoids and amarogentin were identified. Activities of the xanthones mangiferin (IC₅₀ 16.06 μ g/ml), swerchirin (IC₅₀ 162.84 μ g/ml), decussatin (IC₅₀ 195.11 μ g/ml), 1-hydroxy-3,5,8-trimethoxy xanthone (IC₅₀ 245.97 μ g/ml), bellidifolin (IC₅₀ 390.26 μ g/ml) were significantly higher than that of silymarin (IC₅₀ 794.62 μ g/ml). Quinic acid (IC₅₀ 2.91 mg/ml), *O*-acetylsalicylic acid (IC₅₀ 48.4 mg/ml), citric acid (IC₅₀ 1.77 mg/ml), *D*-malic acid (IC₅₀ 14.82 mg/ml) and succinic acid (IC₅₀ 38.86 mg/ml) also inhibited the enzyme β -glucuronidase. The findings suggest that constituents, in addition to the xanthones, probably also contribute to the bioactivity of different *Swertia* species by synergistic effect. Further *in vivo* study is required to support the claim.

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Introduction

Liver disease has become a major health issue globally (Byass, 2014). The liver is a vital organ that is involved in maintenance of metabolic functions and helps in the detoxification process by countering several exogenous and endogenous challenges (Kshirsagar et al., 2011). Glucuronidation is a major pathway of phase II xenobiotic biotransformation (de Graaf et al., 2002). Conjugation of toxins with glucuronic acid deactivates potentially damaging compounds and subsequently eliminates them from the body. However, this process becomes limited by the rate of deglucuronidation by β -glucuronidase. Hydrolysis of the glucuronide moiety can be carried out by β -glucuronidase present in most of the tissues, in endocrine and reproductive organs (Dutton, 1980).

Liver damage causes an increase in the level of β -glucuronidase in blood (Pineda et al., 1959), and liver cancer could be related to this enzyme (Mills and Smith, 1951).

β -Glucuronidase inhibitors reduce the carcinogenic potential of toxic compounds normally excreted in bile after glucuronidation (Walaszek et al., 1984). Due to this correlation, β -glucuronidase inhibitors are suggested as potential hepatoprotective agents (Shim et al., 2000). Certain hepatoprotective plant extracts and their constituents are known to inhibit the enzyme, β -glucuronidase (Joshi and Sanmugapriya, 2007). Silymarin (a mixture of flavonolignans), the commercial plant derived β -glucuronidase inhibitor (Kim et al., 1994), is used to treat liver disorders and also certain cancers (Dixit et al., 2007). But it has poor bioavailability (Dixit et al., 2007). Silymarin has certain other limitations related to gastrointestinal tract like bloating, dyspepsia, nausea, irregular stool and diarrhoea. It also produced pruritus, headache, exanthema, malaise, asthenia, and vertigo (Pradhan and Girish, 2006). Hence, search for glucuronidase inhibitory compounds from medicinally important traditional plants that are earlier reported to be hepatoprotective is necessary.

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Plants of the genus *Swertia*, Gentianaceae, are well recognized in literature as important medicinal herb having an array of biological and therapeutic properties (Negi et al., 2011). Hepatoprotective and anti-hepatotoxic activity of *Swertia* sp. have already been established in Ayurvedic medical system and validated scientifically in animal system (Mukherjee et al., 1997; Karan et al., 1999; Reen et al., 2001). *Swertia chirayita* (Roxb.) Buch.-Ham ex C.B. Clarke, considered to be the most important species of *Swertia* reported from India, for its medicinal properties, has been considered as critically endangered plant (Pant et al., 2000; Joshi and Dhawan, 2005; Bhargava et al., 2009). This plant is substituted by other species like *S. decussata* Nimmo ex C.B. Clarke and *S. bimaculata* (Siebold & Zucc.) Hook. f. & Thomson ex C.B. Clarke (Chopra et al., 1956; Phoboo et al., 2010). Metabolites such as terpenoids, flavonoids, iridoid glycosides and xanthenes are considered as active constituents of *Swertia* sp., xanthenes being the main active secondary metabolite (Brahmachari et al., 2004; Nag et al., 2015).

In a previous study antioxidant, anti-glycosidase and anti-acetylcholinesterase properties of *S. chirayita* and the two substitutes were reported from the laboratory (Nag et al., 2015). Although the hepatoprotective property of *S. chirayita* is well known, the mode of action for hepatoprotection has not yet been studied. The active principles for hepatoprotection are also not known. β -Glucuronidase inhibitory properties of the extracts of these plants would further validate their hepatoprotective property. So, the aim of the study was to compare metabolite profile and β -glucuronidase inhibitory activity of three important species of *Swertia* i.e. *S. chirayita*, *S. decussata* and *S. bimaculata* in order to identify the active constituents.

Materials and methods

Plant material

Leafy shoots of three species of *Swertia*, Gentianaceae, namely *Swertia chirayita* (Roxb.) Buch.-Ham ex C.B. Clarke (Voucher no. Bot 332S-1), *S. bimaculata* (Siebold & Zucc.) Hook. f. & Thomson ex C.B. Clarke (Voucher No. Bot 332S-2) were collected from Darjeeling Himalayas. The third species *S. decussata* Nimmo ex C.B. Clarke (Voucher No. Bot 332S-3) was collected from the Western Ghats, India. Voucher specimens are available in the Department of Botany, University of Calcutta. The two names *S. chirayita* and *S. decussata* are unresolved as per IPNI (International Plant Names Index).

Chemicals and reagents

β -Glucuronidase (ex. bovine liver), 4-nitrophenyl- β -D-glucuronide; methoxyamine hydrochloride, *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane (MSTFA), adonitol and FAME (Fatty Acid Methyl Ester) markers were obtained from Sigma-Aldrich (St. Louis, MO, USA); HPLC grade acetonitrile, formic acid, water, methanol, chloroform and pyridine from Merck Specialities Private Limited (Mumbai, India). Six standard compounds: mangiferin, amarogentin, bellidifolin, swerchirin/methylbellidifolin, decussatin and 1-hydroxy-3, 5, 8-trimethoxy xanthone were available in the laboratory.

Sample preparation

Methanolic extracts of the leafy shoots of *Swertia* sp. were prepared by refluxing dried, ground materials with methanol for 5 h. For each plant material, the filtrate, after extraction, was evaporated to dryness under reduced pressure. Different concentrations of the methanolic extract and that of reference compounds were

used for studying the enzyme inhibition activity *in vitro* as well as for HPLC and GC/MS analysis.

Assay for β -glucuronidase inhibition

β -Glucuronidase inhibition assay was carried out as per the method of Kim et al. (1999) with modification. In brief, 100 μ l of β -glucuronidase (986.4 units/ml in 0.1 M phosphate buffer, pH 7.0) and 340 μ l of test solution/reference standard of various concentrations in 0.1 M phosphate buffer (pH 7.0) were pre-incubated at 37 °C for 15 min. Following the pre-incubation, 60 μ l of *p*-nitrophenyl- β -D-glucuronide (3.15 mg/ml in 0.1 M phosphate buffer, pH 7.0) was added and incubated at 37 °C for 50 min. The colour developed was read at 405 nm in spectrophotometer. Controls were devoid of test samples. The percent inhibition was calculated as follows:

$$\text{Inhibition (\%)} = \left[\frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \right] \times 100.$$

HPLC analysis

The HPLC analysis was performed on an Agilent 1260 (Agilent Technologies, USA) HPLC system consisting of a quaternary pump, a column temperature controller and a diode-array detector (DAD). The analytical column (Agilent Eclipse plus C18, 100 \times 4.6 mm, 3.5 μ m) was used for the analysis. The mobile phase was composed of solvent A (acetonitrile) and solvent B (0.1% formic acid aqueous, v/v). The linear gradient programme followed was: 10% A at 0 min, 30% A at 20 min, 60% A at 35 min and 80% A at 45 min (Du et al., 2012). The flow rate was 0.7 ml/min. 20 μ l aliquots were injected. UV spectra of the peaks were recorded from 190–400 nm over a range of 8 different UV wavelengths (210, 214, 230, 250, 254, 260, 273, and 280 nm respectively).

GC/MS analysis

GC-MS analysis was performed using Agilent 7890 A GC [software driver version 4.01 (054)] equipped with 5795C inert MSD with Triple Axis Detector. The column used for quantification analysis was HP-5MS capillary column [Agilent J & W; GC Columns (USA)] of dimensions 30 m \times 0.25 mm \times 0.25 μ m. The method of Kind et al. (2009) was followed after modification (Das et al., 2016). The analysis was performed under the following oven temperature programme: oven ramp 60 °C (1 min hold), to 325 °C at 10 °C/min, held for 10 min before cool-down producing a run time of 37.5 min. The injection temperature was set at 250 °C, the MSD transfer line at 290 °C and the ion source at 230 °C. Helium was used as the carrier gas (flow rate 0.723 ml/min; carrier linear velocity 31.141 cm/s). The dried crude extract was derivatized using methoxyamine hydrochloride and MSTFA to increase the volatility of the metabolites. A mixture of internal Retention Index (RI) markers (methyl esters of C8, C10, C12, C14, C16, C18, C20, C22, C24 and C26 linear chain length fatty acids) (2 μ l) was added to each sample. Derivatized samples were injected *via* split mode (split ratio 10:1) on to the column. Mass spectra ranging 30–500 *m/z* were recorded. Automated mass spectral deconvolution and identification system (AMDIS) was used to deconvolute and identify chromatographic peaks. The metabolites were identified by comparing the fragmentation patterns of the mass spectra, retention times (RT) and retention indices (RI) with entries of mass spectra, RT and RI in Agilent GC-MS Metabolomics RTL Library (2008) (Agilent Technologies, USA). The relative response ratios of all the metabolites were calculated after normalizing the peak areas of the metabolites by extract dry weight and the peak area of the internal standard.

Statistical analysis

Each experiment was repeated four–five times. Percentage inhibition in activity is presented as mean \pm standard deviation. Regression equations were prepared from the concentrations of the extracts and percentage inhibition of enzyme activity. IC_{50} (concentration of sample required to inhibit 50% enzyme activity) values were calculated from these regression equations. The differences in activity were calculated by Tukey's and Bonferroni's tests.

Results and discussions

The entire plant of *S. chirayita* is used in traditional system of medicine (Joshi and Dhawan, 2005). During the present study leafy shoots could be collected. So methanolic extracts of the leafy shoots of three species of *Swertia* were tested for β -glucuronidase inhibitory activity. Concentration required for 50% inhibition of enzyme activity (IC_{50} value) for silymarin was detected to be $794.62 \pm 10.01 \mu\text{g/ml}$. So, initially, β -glucuronidase inhibition activities of all three species were measured at $500 \mu\text{g/ml}$. It was observed that *S. bimaculata* had lowest activity at the tested concentration (Fig. 1). The activities of *S. chirayita* and *S. decussata* were higher with no significant differences in activity between them. So the IC_{50} values of the two species *S. chirayita* and *S. decussata* were determined. The extracts inhibited the enzyme in a dose dependent manner. It was observed that *S. chirayita* had lower IC_{50} value between the two species indicating stronger activity (Fig. 2). IC_{50} values of both the extracts were significantly

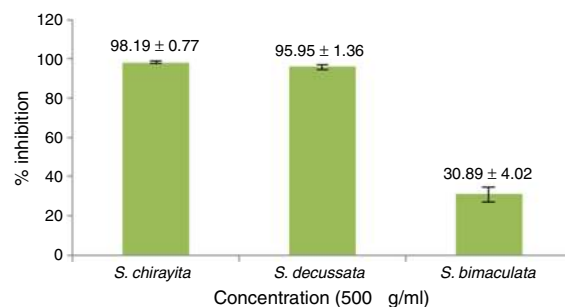


Fig. 1. Comparison of β -glucuronidase inhibitory activity of *Swertia* sp.

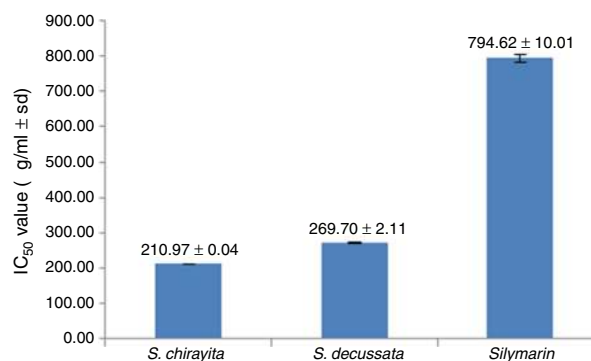


Fig. 2. Comparison of enzyme activity.

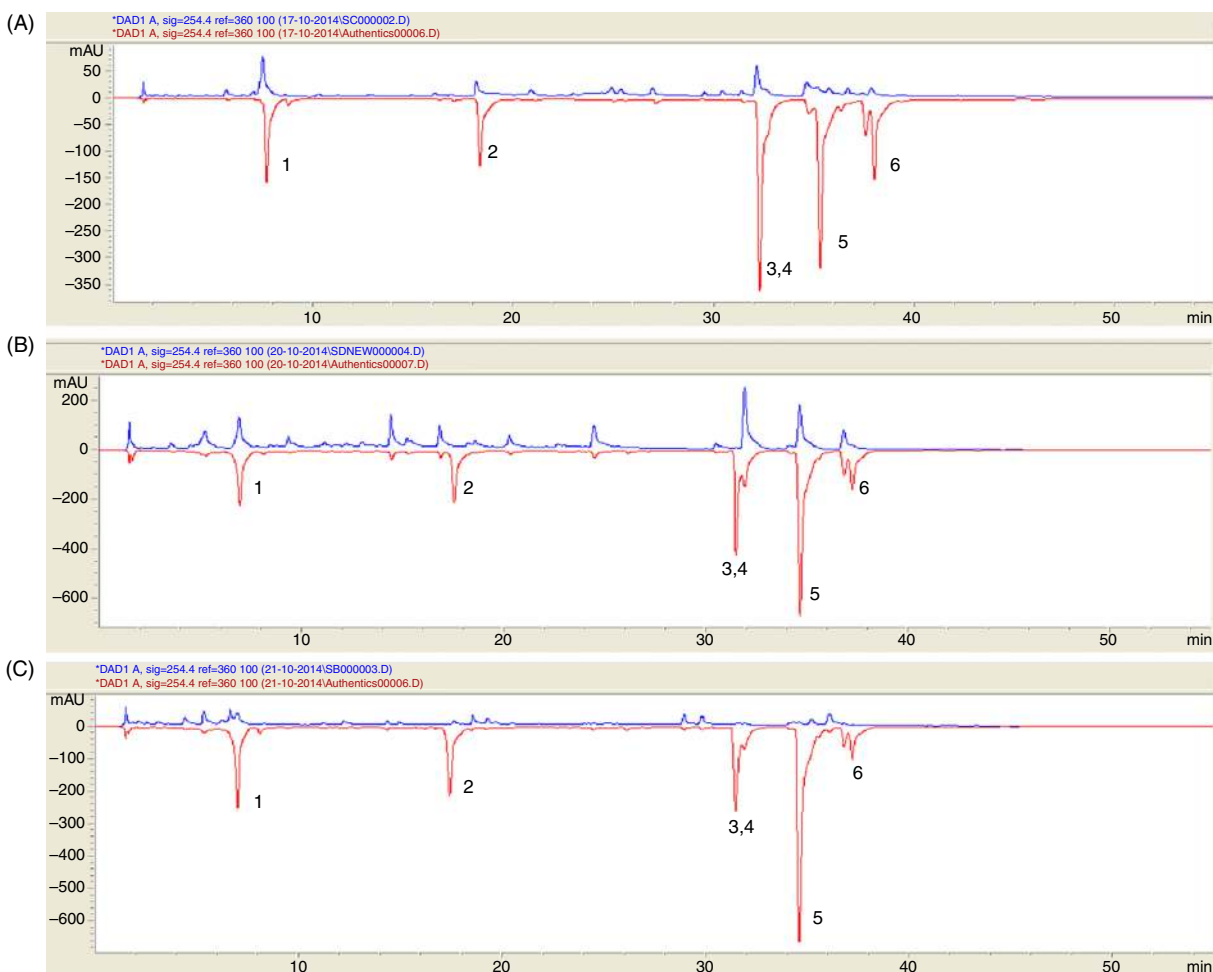


Fig. 3. HPLC chromatogram of (A) *Swertia chirayita*; (B) *Swertia decussata*; (C) *Swertia bimaculata* compared with reference compounds as mirrored images. 1: mangiferin; 2: amarogentin; 3, 4: bellidifolin and 1-hydroxy-3,5,8-trimethoxy xanthone; 5: decussatin; 6: swerchirin.

Table 1
Comparison of HPLC identified metabolites among three species of *Swertia* in elution order.

Standard/reference compounds	<i>S. chirayita</i>	<i>S. decussata</i>	<i>S. bimaculata</i>
	Relative response ratio per g extract		
Mangiferin	1.084 ± 0.39	0.758 ± 0.17	0.209 ± 0.02
Amarogentin	0.376 ± 0.07	–	0.040 ± 0.01
Bellidifolin + 1-Hydroxy-3,5,8-trimethoxy xanthone	0.661 ± 0.22	1.463 ± 0.31	0.043 ± 0.01
Decussatin	0.159 ± 0.02	1.130 ± 0.21	0.066 ± 0.01
Swerchirin	0.233 ± 0.06	0.452 ± 0.17	–

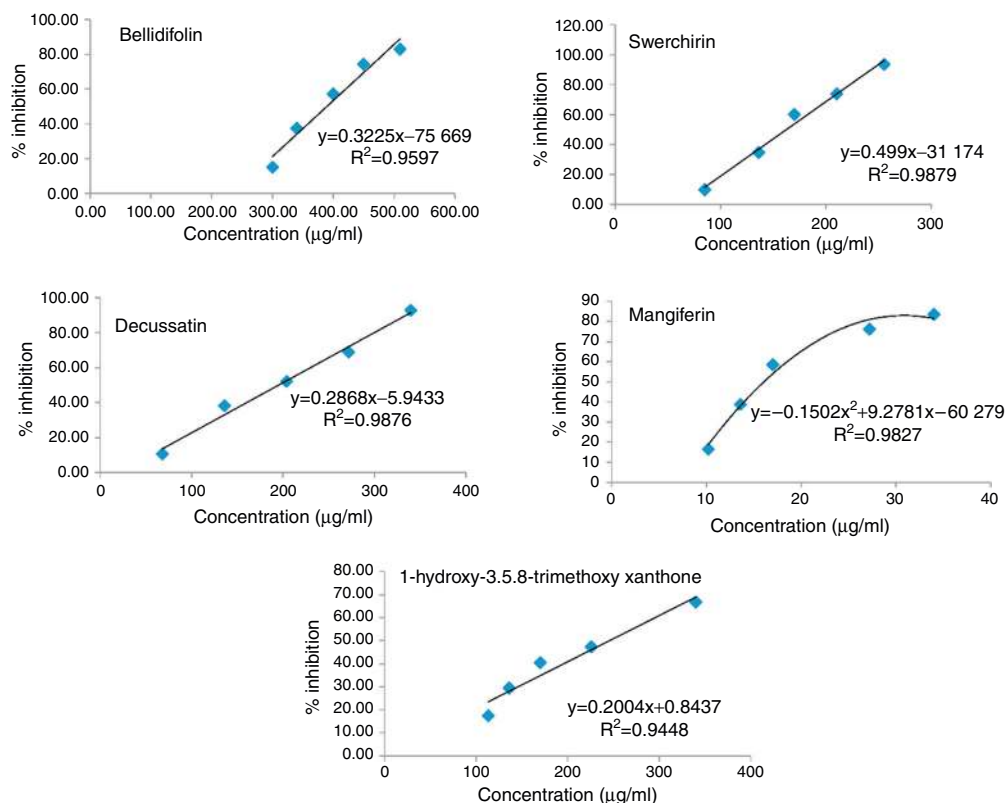


Fig. 4. β -Glucuronidase inhibition by the xanthones.

lower than that of silymarin. The bioactivity of a plant is due to the phytoconstituents present in it. For a comparative study of the metabolite profile in the three species, identification and semiquantitative analyses of the metabolites were performed by HPLC with photodiode array detection and GC–MS following a metabolomics approach. HPLC profile of the three species of *Swertia* (Fig. 3) showed the confirmed presence of three xanthones (swerchirin, decussatin, mangiferin) and the iridoid amarogentin. Bellidifolin and 1-hydroxy-3,5,8-trimethoxy xanthone could not be separated from each other by HPLC as their retention time (RT) and absorbance were same. Semiquantitative comparison of the normalized peak area revealed that mangiferin was present in maximum concentration in *S. chirayita*, followed by *S. decussata* and *S. bimaculata*. Amarogentin and swerchirin were not detected in *S. decussata* and *S. bimaculata* respectively. Decussatin was found to be in maximum amount in *S. decussata*. A comparative account of the HPLC identified metabolites has been represented in Table 1. GC–MS based metabolomics approach helped in identification of 72 compounds from the methanol extract of three species of *Swertia*. Five amino acids, twenty one organic acids, one inorganic acid, eight fatty acids, sixteen phenols, eight sugars, seven sugar alcohols, five terpenoids and one other organic compound (porphine) were identified. A semi quantitative comparison, based on the relative

response ratio per g extract, of the identified metabolites has been represented in Table 2. *S. chirayita* presented maximum number of metabolites followed by *S. bimaculata* and *S. decussata* respectively.

Five xanthones and the iridoid amarogentin, isolated from *S. chirayita* previously (Nag et al., 2015), were tested for their β -glucuronidase inhibitory property. The activities of the compounds were compared with that of silymarin. The activities of the xanthones tested were proportional to their concentrations (Fig. 4). All the xanthones showed inhibitory activities significantly higher than that of the commercial drug (Fig. 5).

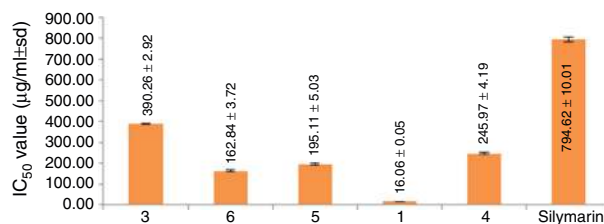


Fig. 5. Comparison of β -glucuronidase inhibitory activity of *Swertia* xanthones with silymarin. 1: Mangiferin; 3: Bellidifolin; 4: 1-Hydroxy-3,5,8-trimethoxy xanthone; 5: Decussatin; 6: Swerchirin.

Table 2
Comparative metabolic profile of three species of *Swertia* using GC/MS.

Metabolites	Relative response ratio per g extract		
	<i>Swertia chirayita</i>	<i>Swertia decussata</i>	<i>Swertia bimaculata</i>
<i>Amino acids</i>			
N-Ethylglycine	1.13 ± 1.09	–	–
N-Acetyl-L-glutamic acid	15.73 ± 2.85	–	21.72 ± 9.41
L-Glutamic acid (dehydrated)	14.43 ± 1.50	1.57 ± 0.71 ^b	–
L-Pyroglutamic acid	–	–	17.12 ± 3.46
Allantoin	37.14 ± 8.73	–	–
<i>Organic acids</i>			
L-(+) Lactic acid	28.97 ± 2.06	25.03 ± 7.83	137.05 ± 14.21 ^a
Glycolic acid	6.83 ± 0.31	2.74 ± 3.95	12.21 ± 0.83 ^a
Oxalic acid	0.98 ± 0.31	–	4.00 ± 2.27 ^a
Malonic acid	3.82 ± 0.91	–	2.88 ± 1.66
Maleic acid	2.59 ± 0.29	2.21 ± 0.45	1.75 ± 1.00
Succinic acid	86.36 ± 2.13	1.62 ± 0.08 ^b	98.39 ± 7.51 ^a
Glyceric acid	90.34 ± 2.72	4.77 ± 0.82 ^b	67.42 ± 3.79 ^b
Fumaric acid	103.78 ± 7.32	7.43 ± 1.57 ^b	18.97 ± 3.81 ^b
Citraconic acid	1.03 ± 0.22	–	0.34 ± 0.25 ^b
Citramalic acid	3.79 ± 0.92	–	–
Mandelic acid	1.05 ± 0.38	–	0.84 ± 0.19
D-Malic acid	705.76 ± 25.78	6.08 ± 0.58 ^b	296.55 ± 44.42 ^b
Adipic acid	–	–	0.76 ± 0.66
Citric acid	19.33 ± 4.44	–	17.68 ± 11.19
Gluconic acid lactone	144.13 ± 14.48	41.30 ± 5.64 ^b	76.96 ± 8.32 ^b
Gluconic acid	15.29 ± 7.38	–	–
2-Isopropylmalic acid	1.51 ± 0.19	–	–
3-Hydroxy-3-methylglutaric acid (dicrotalic acid)	3.29 ± 0.13	–	–
Ribonic acid-gamma-lactone	22.24 ± 35.46	–	–
4-Guanidinobutyric acid	0.93 ± 0.30	–	–
Nicotinic acid	2.04 ± 0.87	–	2.74 ± 0.81
<i>Inorganic acid</i>			
Phosphoric acid	14.97 ± 2.36	3.99 ± 0.45 ^b	5.66 ± 1.15 ^b
<i>Fatty acids</i>			
Lauric acid	1.26 ± 0.13	1.44 ± 0.73	6.16 ± 2.69 ^a
Behenic acid	8.71 ± 1.06	–	14.63 ± 3.04 ^a
Myristic acid	–	–	6.79 ± 1.82
Palmitic acid	109.52 ± 11.08	94.42 ± 15.02	247.32 ± 10.48 ^a
Linoleic acid	26.08 ± 5.82	3.69 ± 2.31 ^b	27.39 ± 7.70 ^c
Oleic acid	32.02 ± 9.41	4.93 ± 3.89 ^b	20.48 ± 16.95
Stearic acid	64.63 ± 7.34	64.87 ± 10.13	116.75 ± 13.21 ^a
Arachidic acid	4.13 ± 0.99	–	20.27 ± 4.24 ^a
<i>Phenols</i>			
Benzoic acid	–	–	1.97 ± 0.22
O-Acetylsalicylic acid	–	–	3.60 ± 0.35
4-Hydroxybenzoic acid	9.44 ± 0.72	–	4.75 ± 0.12 ^b
2,3-Dihydroxybenzoic acid	41.22 ± 0.14	22.78 ± 1.75 ^b	112.23 ± 2.24 ^a
4-Hydroxy-3-methoxybenzoic acid	56.08 ± 1.65	1.48 ± 0.40 ^b	47.02 ± 0.72 ^b
Gentisic acid	–	3.58 ± 1.76	33.83 ± 6.61 ^c
Shikimic acid	1.47 ± 0.21	–	–
3,4-Dihydroxybenzoic acid	43.59 ± 16.66	–	8.83 ± 0.67 ^b
Quinic acid	2.06 ± 0.33	5.29 ± 2.96	26.20 ± 0.77 ^a
Coniferyl alcohol	6.90 ± 1.95	–	22.27 ± 9.60 ^a
4-Hydroxycinnamic acid	–	11.30 ± 5.60	–
Ferulic acid	–	7.76 ± 6.08	–
Sinapyl alcohol	–	–	3.46 ± 1.96
Caffeic acid	0.86 ± 0.09	1.44 ± 0.72	–
Neohesperidin	–	0.95 ± 0.34	–
Isoquercitrin	59.38 ± 8.10	–	–
<i>Sugars</i>			
Methyl-β-D-galactopyranoside	105.99 ± 1.29	26.71 ± 14.48 ^b	45.95 ± 40.50 ^b
Sucrose	1482.49 ± 114.56	27.04 ± 4.29 ^b	1682.28 ± 1055.76 ^c
Lactose	46.34 ± 43.84	–	–
D-(+)-Trehalose	248.92 ± 66.71	22.69 ± 7.72 ^b	86.93 ± 15.65 ^b
Raffinose	2.46 ± 1.91	–	6.39 ± 5.06
Melezitose	3.70 ± 1.99	3.19 ± 0.28	4.55 ± 3.22
D-(+)-Melezitose	1.98 ± 0.83	86.62 ± 100.91	–
Adenosine	9.66 ± 7.90	–	6.95 ± 1.24
<i>Sugar alcohols</i>			
Glycerol	108.61 ± 1.07	58.94 ± 1.09 ^b	563.47 ± 10.97 ^a
Glycerol 1-phosphate	2.98 ± 0.26	–	–
D-Threitol	58.63 ± 4.42	7.61 ± 1.60 ^b	4.60 ± 0.93 ^b
Arabitol	81.81 ± 24.82	47.28 ± 17.36	–

Table 2 (Continued)

Metabolites	Relative response ratio per g extract		
	<i>Swertia chirayita</i>	<i>Swertia decussata</i>	<i>Swertia bimaculata</i>
D-Mannitol	533.42 ± 19.80	12.45 ± 1.06 ^b	66.75 ± 1.01 ^b
D-Sorbitol	199.74 ± 2.90	115.11 ± 18.17 ^b	323.37 ± 47.73 ^a
Galactinol	37.99 ± 6.29	–	–
Terpenoids			
Phytol	–	–	3.44 ± 0.66
Loganin	–	105.87 ± 12.81 ^c	34.05 ± 7.11
Palatinitol	28.05 ± 15.45	–	8.68 ± 4.87
Stigmasterol	39.26 ± 5.79	3.15 ± 2.76 ^b	42.25 ± 7.60
Lanosterol	–	–	35.94 ± 7.56
Macrocyclic organic compound			
Porphine	6.41 ± 4.38	–	–

–, not detected.

^a Significantly higher than *S. chirayita*.

^b Significantly lower than *S. chirayita*.

^c Significantly higher between *S. decussata* and *S. bimaculata*.

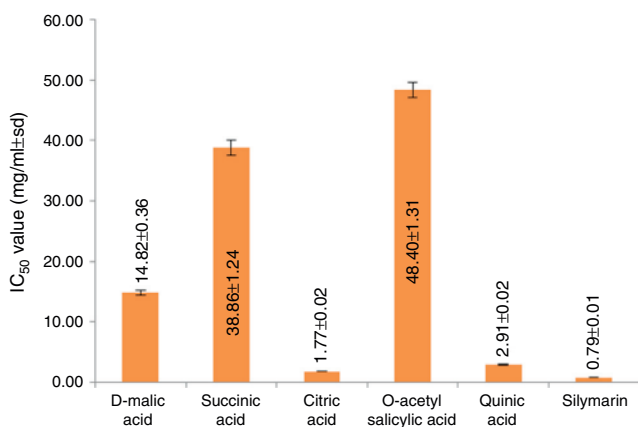


Fig. 6. Comparison of β -glucuronidase inhibitory activity of organic and phenolic acids with respect to silymarin.

Mangiferin showed the best β -glucuronidase inhibition with an IC_{50} value of $16.06 \pm 0.05 \mu\text{g/ml}$ or 0.038 mM followed by swerchirin (IC_{50} $162.84 \pm 3.72 \mu\text{g/ml}$ or 0.565 mM), decussatin (IC_{50} $195.11 \pm 5.03 \mu\text{g/ml}$ or 0.646 mM), 1-hydroxy-3,5,8-trimethoxy xanthone (IC_{50} $245.97 \pm 4.19 \mu\text{g/ml}$ or 0.814 mM) and bellidifolin (IC_{50} $390.26 \pm 2.92 \mu\text{g/ml}$ or 1.424 mM). However, the bitter iridoid compound, amarogentin did not show any enzyme inhibitory activity. Out of 72 metabolites identified by GC–MS, nine compounds, available in the laboratory, were tested for their β -glucuronidase inhibitory activity. These were succinic acid, D-malic acid, citric acid, O-acetylsalicylic acid, 4-hydroxybenzoic acid, quinic acid, 4-hydroxycinnamic acid, sucrose and glycerol. 4-Hydroxybenzoic acid, 4-hydroxycinnamic acid, sucrose and glycerol did not have any β -glucuronidase inhibitory activity. Remaining five compounds inhibited β -glucuronidase in a dose-dependent manner. Comparison of their activities with respect to silymarin had been illustrated in Fig. 6. Among these, citric acid (IC_{50} $1.77 \pm 0.02 \text{ mg/ml}$) and quinic acid (IC_{50} $2.91 \pm 0.02 \text{ mg/ml}$) showed activity close to silymarin (IC_{50} $0.79 \pm 0.01 \text{ mg/ml}$).

Xanthenes had already been reported to possess a range of pharmacological actions (Peres et al., 2000). *S. chirayita*, *S. decussata* and *S. bimaculata* are considered to be a natural source of tetraoxygenated xanthenes (Peres et al., 2000). Mangiferin, a xanthone-C-glycoside, had previously been reported to possess antioxidant (Nag et al., 2015); anti-diabetic and antitumour activities to name a few (Suryawanshi et al., 2006). Bellidifolin and swerchirin had been found to be potent hypoglycemic agent (Bajpai

et al., 1991; Basnet et al., 1995). In addition, swerchirin, had also been reported to be hepatoprotective (Hajimehdipoor et al., 2006) on paracetamol-induced hepatotoxicity in mice models. Several studies that had been carried out to advocate the hepatoprotective and anti-hepatotoxic property of this genus credits this attribute to the xanthone content present in extract of the plant. The five xanthenes that had been considered in our study showed good β -glucuronidase inhibition in comparison to the commercial drug, silymarin, thereby proposing a possible mechanism of hepatoprotective action via β -glucuronidase inhibition. These findings had not been reported earlier in literature. The present study also reveals that some organic acids viz., succinic acid, D-malic acid, citric acid and phenolic compounds viz., O-acetylsalicylic acid and quinic acid have β -glucuronidase inhibitory properties. Citric acid was reported earlier to reduce lipopolysaccharide induced liver injury and oxidative stress (Abdel-Salam et al., 2014). So, β -glucuronidase inhibition property of the constituents present in *Swertia* sp. may be a mechanism for hepatoprotective activity of these plants. Further *in vivo* study is required in this regard.

Conclusion

The properties of three *Swertia* sp. e.g. *S. chirayita*, *S. decussata* and *S. bimaculata* to inhibit β -glucuronidase, a mechanism for hepatoprotection, were assessed and the contributory constituents had been identified. Several xanthenes were identified to be major components to have significantly higher β -glucuronidase inhibition properties than that of silymarin. Metabolites other than the xanthenes, probably also contribute to the bioactivity of different *Swertia* species by synergistic effect. The present findings suggest that β -glucuronidase inhibition may be one of the mechanisms for the hepatoprotective property of *Swertia* sp. Further *in vivo* study is required to support the claim.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals during the study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contributions

SK performed extraction, chromatographic analysis, bioactivity studies and preparation of the manuscript. GN performed extraction and some analysis. BD provided idea, project plan and subsequently preparation of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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