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Phytochemical Studies on *Swertia cordata* (G. Done) Clark and Comparative Chromatographic Evaluation of Five Different Species of *Swertia*

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Swertia cordata (G. Done) Clark is an important species of the genus Swertia (Family Gentianaceae) reputed for its medicinal properties in Ayurveda. The present study reports the isolation of two xanthones, 1-hydroxy-2,3,7-trimethoxyxanthone ***(1),*** *1-hydroxy-2,3,4,7-tetramethoxyxanthone* ***(2)*** *and one triterpenoid, lupeol* ***(3)*** *from S. cordata. The compounds* ***(1)*** *and* ***(2)*** *are being reported for the first time from S. cordata. Further, chromatographic comparison of five different species of Swertia viz. S. angustifolia Buch Ham. ex D. Don, S. chirata Buch Ham, S. cordata (G. Done), S. lurida (D. Don ex G. Done) C. B. Clarke and S. purpurascens (D. Don) A. Wall ex E. D. Clarke was made on the basis of TLC fingerprint profile to know how closely these species are related and to mark the similarities / dissimilarities between different Swertia species.*

Key words: *Swertia* species, xanthones, TLC fingerprint comparison

INTRODUCTION

The genus *Swertia* belongs to family Gentianaceae which is known to have about 700 species and 80 genera. The plants of this family are annual or perennial herbs. About 150 species are distributed in Asia, Europe, North America and Africa. Nearly 75 species are known from China only.[1] Most of the species found in India grow at high altitude in the temperate Himalayas from Kashmir to Bhutan and also in the Khasia and Western Ghat Hills.[2] *Swertia chirata* Buch Ham. is an official drug of the Indian Pharmacopoeial List 1946 and is one of the most reputed plants of Ayurveda.[3] The bitterness, anthelmintic, hypoglycemic and antipyretic properties are attributed to amarogentin, swerchirin, swertiamarin and other active principles of the herb.[4] The herb is reported as potential hepatoprotective and antimalarial.[5-7] The decoction of whole plant and root of *S. angustifolia* Buch Ham. ex D. Don is used for the treatment of malarial fevers, while root juice gives relief from cold and cough. *S. angustifolia* contain 14 tetraoxygenated and 5 pentaoxygenated xanthones and xanthone 1-*O*- glucosides.[8] *S. purpurascens* (D. Don) A. Wall ex E. D. Clarke is used in the Indian System of Medicine as a substitute for *S. chirata*.[1] The whole plant has been shown to contain 5 tetraoxygented and 3 pentaoxygenated xanthones.[9] *S. cordata* (G. Don) C.B.Clarke is used as an alternative febrifuge, anthelmintic and bitter tonic. Four xanthones, 1-hydroxy-3,5,7,8-tertamethoxyxanthone,1,7-dimethoxy-3,5,8-trimethoxyxanthone,1,3,6,7-tetrahydroxy-2-glucosylxanthone (mangiferin) and 1,5-dihydroxy-8-*O*-glucosyl-3-methoxyxanthone (swertianolin) have been reported form *S. cordata*.[10,11] It has shown antihepatotoxic activity against CCl_4 and paracetamol (acetaminophen) toxicity in primary monolayer cultures of rat hepatocytes.[12] *S. lurida* (D. Don ex G. Don) C. B. Clarke is reported to contain bitter compounds such as iridoids and seco-iridoids.[13]

The phytochemical investigations of the genus *Swertia* have yield approximately 200 compounds with varying structural patterns till date. Xanthones, iridoids/secoiridoids and triterpenoids constitute the major classes of compounds reported from the genus.[13] Xanthones are class of tricyclic compounds characterized by a dibenzo-γ-pyrone nucleus. The prefix 'xanth' means 'yellow' colour of these compounds and '-one' is from their 'keto' nature. The xanthones bear a close structural relationship to other naturally occurring γ-pyrone derivatives like flavonoids and chromones.[14] Most abundant xanthones are tetraoxygented and

pentaoxygented with additional oxygenation occurring at C-8, C-6, C-4 and C-2 of dibenzo-γ-pyrone nucleus.[15] In the present study, phytochemical investigations of *S. cordata* were undertaken and a

comparative profile of five different species of *Swertia* was developed to describe how closely these species are related. The study is proposed to be of significance in establishing chemotaxonomic relationship between different *Swertia* species.

MATERIAL AND METHODS

Plant material

The whole plant of *Swertia cordata* was collected from Sirkunda Devi (Mussoorie, Uttarakhand, India) situated at an altitude of 2050 meters, in the month of September 2007. The plant material was authenticated on the basis of morphological characters reported in the standard text and by comparison with reference herbarium specimens available at the Museum-cum-Herbarium of University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh. A voucher specimen no (1459) has been deposited at the Institute. The authenticated raw material of other species of *Swertia viz. S. angustifolia, S. chirata, S. lurida* and *S. purpurascens* available vide reference number 1462, 1458, 1463 and 1144 respectively was taken from Museum-cum-Herbarium of University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh for the present study.

Extraction and fractionation of the plant material

The extraction of *S. cordata* was carried out at room temperature by macerating the coarsely powdered whole plant material (700 g) with methanol and occasionally shaking the contents. The extract was concentrated under vacuum in a rotary evaporator (Eyela, NE-10, Japan). The residue from methanolic extract was sequentially partitioned with hexane, chloroform and ethyl acetate to separate compounds of different polarities. The hexane and chloroform soluble portion of methanolic extract showing maximum number of spots on TLC were processed for the isolation of xanthones. The ethyl acetate soluble fraction was not taken up further for fractionation and isolation of the compounds as the weight of residue was negligible.

Isolation of compounds

Column chromatography was used for fractionation of the crude extracts and isolation of pure compounds. Silica gel (60-120 and 100-200 mesh, S.D Fine-chem. Ltd.) was employed as an adsorbent. The elutions were carried out in a gradient fashion using increasing order of polarity of the eluting solvent. Thin layer chromatography was performed in laboratory using silica gel G. The number and position of spots were visualized by spraying the plate with 10 % aqueous KOH/anisaldehyde-sulphuric acid reagent and/or observing the plate under ultraviolet light. Medium Pressure Liquid Chromatography (MPLC, Buchi C-615 pump) or preparatrive TLC was employed for processing fractions obtained through column chromatography for isolation of pure compounds. The elutions in MPLC were carried out in a gradient fashion using increasing order of polarity of the eluting solvent maintaining constant flow rate and pressure.

A compound (**1)** was isolated from the chloroform soluble portion (8 g) of methanolic extract. The fraction eluted in chloroform was subjected to preparative TLC using solvent system hexane : ethyl acetate (7:3) for the isolation of compound with R_f value 0.34. The isolate was re-crystallized from a mixture of chloroform and methanol. A compound (**2)** was isolated from the hexane soluble portion (8 g) of methanolic extract. The fraction eluted in chloroform was further subjected to medium pressure liquid chromatography and fraction eluted in hexane containing 67-72% chloroform was processed through preparative TLC using solvent system hexane : ethyl acetate (7:3) for the isolation of compound with R_f value 0.70. The isolate was re-crystallized from a mixture of chloroform and methanol. A compound (**3)** was isolated from hexane soluble portion of methanolic extract. The fraction eluted in hexane : chloroform (20:80) was also subjected to medium pressure liquid chromatography. The fraction eluted in hexane containing 82-90 % chloroform on rechromatography yielded the pure isolate (**3)**.

Compound (1)

Yellow crystalline compound, 20 mg (0.00028 %); m.p. 165-167^{o}C; TLC, R_f 0.38 (Toluene : Acetone :: 9.5:0.5); UV λ_{max} nm 298, 240 and 206; IR (KBr)υ_{max} 3435, 2922, 2853, 1651, 1577 and 1306; ^{1}H-NMR (400 MH_z, $CDCl_3$) δ 12.76 (1H, s), δ 7.85 (1H, dd, J = 8, 1.6 Hz), δ 7.32 (1H, d, J = 8 Hz), δ 7.24 (1H, d, J= 1.6 Hz), δ 6.64 (1H, s), δ 4.04 (3H, s), δ 3.97 (3H, s), δ 3.92 (3H, s).

Compound (2)

Yellow crystalline compound,10 mg (0.00014 %); m.p. 114-115^{o}C; TLC, R_f 0.50 (Toluene : Acetone :: 9.5:0.5); UV λ_{max} nm 380, 298, 262, 230 and 206; IR(KBr) υ_{max} 3446, 2925, 2852, 1643, 1601 and 1266; ^{1}H-NMR (400 MH_z, $CDCl_3$) δ 12.66 (1H, s), δ 7.61 (1H, d, J = 3.2 Hz), δ 7.51 (1H, d, J = 9.2 Hz), δ 7.36 (1H, dd, J = 9.2, 3.2 Hz), δ 4.15 (3H, s), δ 3.96 (3H, s), δ 3.95 (3H, s), δ 3.92 (3H, s).

Compound (3)

White amorphous compound, 58.4 mg (0.00078 %); m.p. 205-207^{o}C; TLC, R_f 0.34 (Toluene : Acetone :: 9:1); UV λ_{max}nm 207; IR(KBr) υ_{max} 3361, 2941, 1646, 1460, 1379, 1035 and 884; ^{1}H-NMR(400 MH_z, $CDCl_3$) δ 5.19 (1H, brs), δ 4.57 (1H, brs), δ 3.20 (1H, dd, J= 10.8, 4.4 Hz), δ 1.07 (1H, s), δ 1.03 (1H, s), δ 0.96 (1H, s), δ 0.95 (1H, s), δ 0.83 (1H, s), δ 0.80 (1H, s), δ 0.79 (1H, s).

Spectroscopic methods

The UV spectra were obtained in methanol on Perkin Elmer Lambda-15 Spectrophotometer, IR spectra were obtained in KBr discs on Perkin Elmer 882 Infrared Spectrophotometer,^{1}H-NMR and ^{13}C-NMR spectra were obtained on Bruker 400 MHz/52 MM (Spectrospin and Bruker) and mass spectra were obtained on RKS TOF $MSES^{+}$ 8.70e3 at Regional Sophisticated Instrumentation Centre, Panjab University, Chandigarh.

Thin layer chromatography

The plates for TLC were prepared in the laboratory. Silica gel slurry, prepared in distilled water was spread in 0.25 mm thickness and the plates were activated at 110^{o}C for 30 min in an oven before use and after development in suitable solvents, the number and position of spots were visualized by spraying the plate with 10 % aqueous KOH/anisaldehyde-sulphuric acid reagent or observing the plate under ultraviolet light. For comparative TLC studies all solvents used were of GR grade (E. Merck) and chromatograms were developed on precoated silica gel G plates (E. Merck, alumina base). A large number of solvent systems were tried and used for TLC studies. Of the various solvent systems, toluene : acetone (9.5:0.5) was finally selected for preparing TLC fingerprint profile as it showed maximum resolution and solvent system, hexane : ethyl acetate (7:3) was selected for preparative TLC.

Comparative TLC fingerprint profile of different extracts of various *Swertia* species

About 5 g of powdered material of whole plant of each of the five *Swertia* species *viz. S. angustifolia, S. chirata, S. cordata, S. lurida* and *S. purpurascens* was extracted using soxhlet apparatus for 5 h separately with 100 ml each of methanol, hexane and chloroform to obtain methanol soluble, hexane soluble and chloroform soluble portions. Each extract was concentrated under reduced pressure in rotary vacuum evaporator and reconstituted with appropriate solvents(s) to develop the TLC fingerprint profile of methanolic, hexane and chloroform soluble extracts.

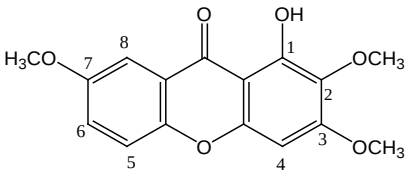
RESULTS AND DISCUSSION

The compound **(1)** isolated from chloroform soluble fraction brightened on spraying with 10% aqueous KOH solution on TLC plate giving characteristic indication of a benzo-*γ*-pyrone moiety which was also supported by UV spectra. The UV spectrum showed absorption maxima at 298, 240 and 206 nm characteristic of xanthones. The IR spectrum showed absorption at 3435, 2922, 1651, 1577, and 1306 cm^{-1} which indicated the presence of hydroxyl, ketone, aromatic ether and aromatic nature of the molecule.^{1}H-NMR showed its tetra-substituted nature because only four of the eight aromatic protons were observed in its spectrum. Three sharp 3H singlets at δ 4.04, 3.97 and 3.92 identified three of the substituents as methoxy

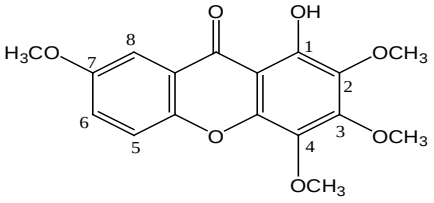
groups. The sharp 3H singlet at δ 4.04 was assigned to methoxy group at C-7 and 3H singlets at δ 3.97 and 3.92 were assigned to methoxyls at C-2 and C-3 respectively, which was supported by literature values. The presence of one double doublet at δ 7.85 (*J*=8 & 1.6 Hz) indicated that one aromatic proton is ortho and

meta coupled with two other aromatic protons. The presence of two doublets at δ 7.32 (*J*=8 Hz) and 7.24 (*J*=1.6 Hz) indicated that these protons are ortho and meta coupled respectively, with proton showing double doublet at δ 7.85. The signals at δ 7.85, 7.32, and 7.24 were assigned to H-6, H-5 and H-8 respectively. One singlet in far downfield region at δ 12.76 was attributed to one hydroxyl group either at C-1 or C-8 and it was due to intramolecular bonding with carbonyl oxygen. One sharp singlet of aromatic proton appeared at δ 6.64 which indicated that one of the two aromatic rings is having three substitutions leaving one position as unsubstituted. This gave a clear indication that hydroxyl must be present at C-1 with C-2 and C-3 occupied by methoxyls leaving C-4 as vacant position. The observed δ values for all the positions were fairly close to the literature values. All these evidences identified compound **(1)** as 1-hydroxy-2,3,7-trimethoxyxanthone which was also confirmed by comparison of our data with reported values for this compound. This is the first report of isolation of 1-hydroxy-2,3,7-trimethoxyxanthone from *S. cordata* and earlier it has been reported from *S. mileensis*.[16]

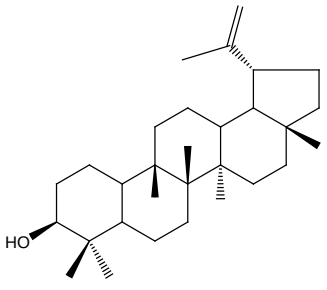
The compound **(2)** isolated from hexane soluble fraction brightened on spraying with 10% aqueous KOH solution. The UV spectrum showed absorption maxima at 380, 298, 262, 230 and 206 nm characteristic of xanthones. The IR spectrum showed absorption at 3446, 1643, 1601 and 1266 cm^{-1} which indicated the presence of hydroxyl, ketone and aromatic ether. ^{1}H-NMR showed its penta-substituted nature because only three of the eight aromatic protons were observed in its spectrum. One singlet in far downfield region at δ 12.66 was attributed to one hydroxyl group either at C-1 or C-8 and it was due to intramolecular bonding with carbonyl oxygen. Four sharp 3H singlets at δ 4.15, 3.96, 3.95 and 3.92 identified four of the substituents as methoxy groups and no singlet for any aromatic proton was obtained which indicated that all the four positions in one of the aromatic ring were substituted by functional groups. The sharp 3H singlet at δ 4.15 was assigned to methoxy group at C-7 and 3H singlet at δ 3.96, 3.95 and 3.92 were assigned to methoxyls at C-2, C-4 and C-3 respectively, which was well supported by literature values. The presence of one double doublet at δ 7.36 (*J*=9.2 & 3.2 Hz) indicated that one aromatic proton is ortho and meta coupled with other aromatic protons. The presence of two doublets at δ 7.51 (*J*=9.2 Hz) and δ 7.61 (*J*=3.2 Hz) indicated that these protons are ortho and meta coupled with proton showing double doublet at δ 7.36. The signals at δ 7.61, 7.51, and 7.36 were assigned to H-8, H-6 and H-5 respectively. The observed δ values for all the positions were fairly close to the literature values. All these evidences identified compound **(2)** as 1-hydroxy-2,3,4,7-tetramethoxyxanthone which was also confirmed by



(1)



(2)



(3)

Figure 1. Structure of isolated compounds

comparison of our data with reported values for this compound. This is the first report of isolation of 1-hydroxy-2,3,4,7-tetramethoxyxanthone from *S. cordata* and earlier it has been reported from *S. mileensis*[16] and *S. chirata*.[17]

The compound **(3)** produced a single pink spot on spraying with anisaldehyde-sulphuric acid reagent followed by heating. UV spectrum showed absorption maxima at 207 nm. IR spectrum showed absorption at 3361, 2941, 1646, 1460, 1379, 1035 and 884 cm^{-1} which indicated the presence of hydroxyl, aromatic C-H stretch and methyl bend of the molecule. ^{1}H-NMR spectrum showed seven signals of tertiary methyl groups at δ 1.07, 1.03, 0.96, 0.95, 0.83, 0.80 and 0.79. The two broad signals at δ 5.19 and 4.57 showed presence of axial and equilateral hydrogen at C-29. The presence of one double doublet at δ 3.26 (*J*=10.8 4.4 Hz) indicated that one aromatic proton is ortho and meta coupled with other aromatic proton which showed presence of hydrogen at H-3. All these evidences led to the identification of the compound **(3)** as lupeol which was also confirmed by comparison of our data with reported values for this compound[18] and by co-TLC with the reference sample available in our laboratory.

The solvent system toluene : acetone (9.5 : 0.5) gave reasonably good resolution for all the species and was used for assessing the profile of xanthones in different species. The fingerprint studies showed both similarities and dissimilarities in the profile of all the extracts. No change in profile was observed when plates were visualized under 254 nm before and after spraying with 10 % aqueous KOH. However, there was apparent difference when plates were observed in white light before and after spraying with 10 % aqueous KOH. A comparative chemical profile of various extracts of different *Swertia* species was made on the basis of fingerprint profile and marker compounds isolated from *S. cordata*.

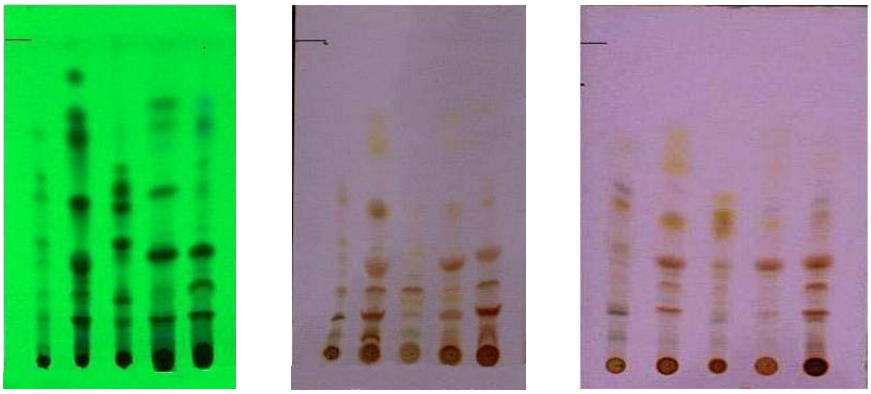


Plate 1: TLC fingerprint profile of methanolic extract (ME, under 254 nm); chloroform extract (CE) and hexane extract (HE) of different *Swertia* species, (a) *S. angustifolia,* (b) *S. chirata,* (c) *S. cordata,* (d) *S. lurida,* (e) *S. purpurascens* after spraying with 10% aqueous KOH solution and visualization under white light. Solvent system, toluene : acetone (9.5: 0.5).

CONCLUSION

The two xanthones 1-hydroxy-2,3,7-trimethoxyxanthone and 1-hydroxy-2,3,7-trimethoxyxanthone have been reported for the first time from *S. cordata*. The two xanthones are also visible in *S. chirata.* A comparative chemical study of five different species of *Swertia* was made on the basis of thin layer chromatography fingerprint profile. The TLC fingerprint studies showed both similarities and dissimilarities in the profile of all the extracts. The maximum number of xanthones was observed in methanol extract of *S. chirata* followed by *S. cordata*, *S. lurida* and *S. purpurascens. S. angustifolia* showed the minimum number of xanthones. The chloroform and hexane extract of *S. chirata* and *S. purpurascens* showed close similarity when observed in white light before and after spraying with 10 %

aqueous KOH. The present study will be of significance to establish the chemotaxonomic relationship between different *Swertia* species.

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