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ABSTRACT

The Uttarakhand state harbours a large number of medicinal plants, which falls within the western Himalayas. This region is known for its diverse and rich floristic wealth. Swertia belongs to the family Gentianaceae. Gentianaceae has been reported to have fourteen genera and forty five species. Out of these, 15 are of Swertia genus found growing in Kumaun Himalayan region at an altitude of 2,000 – 17,500 ft. These species are Swertia chirata Buch-ham, S.allata Buch- ham, S. Peniculate Wall, S. Putchalla Buch-ham, S. angustifollia Buch-ham, S.chordata Royle, S. cuneata Wall, S.Luride Royle, S. tetragona, Clarke. Among all these S.chirata is an important Ayurvedic herband an important ingredientof different herbal medicines. It is also used as a bitter tonic. Recently the hexane extract of this plant has been reported to possess hypoglycaemic activity.

Chemical investigation showed the presence of Xanthones, triterpenoids, flavonoids, Steroids, Sugars and alkaloids in the different species of genus Swertia. S.allata is an important ingredient of Tibetian medicine. The Swertia chirata is a therapeutic plant and its remedial usage has been recognized in the Indian Pharmaceutical codex as well as the British and American Pharmacopoeias. In addition, the curative value of the herb has also been recorded by the ancient Indian herbal medicine system Ayurveda and the otherconventional medical systems.

Keywords: Swertia, Phytoconstituents, Xanthones, Extraction, bioactivity

INTRODUCTION

The medicinal importance of the phytoconstituents have been known to mankind from time immemorial. These natural products obtained from plants are being used as traditional medicines till date. Even after the development of chemically synthesized drugs, many people continue to rely upon conventional methods of treatment. According to WHO more than 80% of the world's population depends on traditional medicines for their primary health needs.

The Himalayan region endows a rich floral diversity and many of them possess medicinal value. One of the traditional medicinal plants of Himalayan region are of genus Swertia. Swertia belongs to the family Gentinaceae. Gentinaceae includes 14 genera and 45 species. Out of these 15 species belong to the genus Swertia and have been found growing in Kumaun Himalayan region at an altitude of 2000 – 17500 ft. These species are Swertia chirata Buch-Ham S. alata Buch Ham, S. purpurascens Wall, Swertia petiolata Royle, Swertia cuneata Wall, Swertia lurida Royle, Swertia peniculata Wall, Swertia putchella Buch-Ham, Swertia speciosa Wall, Swertia angustifolia Buch-Ham, Swertia alternifolia Royle, Swertia chordata Royle, Swertia caerulea Royle, Swertia hookerii, Swertia dialata Clarke var pitora, Swerrtia tetragona, Clarke. Among these Swertia chirata is the most important Ayurvedic herb. It is also known as Nepali Neem as it grows as an annual or biennial herb in the forests of Nepal. Swertia chirata is known as bitter tonic in traditional system of medicine.

The main chemical constituents present in Swertia species are Swertiamarin, Amarogentin, Swerchirin, Mangiferin, Sweroside,Gentianine, Amaroswerin, Oleanolic acid, Swetanoone, Ursolic acid. Phytochemical investigations also confirms the presence of flavonoids, steroids, glycosides, triterpenoids, xanthones and ascorbic acid. Negi et al. evaluated the concentration of trace elements in Swertia chirata which was found in order K>Ca>Fe>Na>Mn>Zn>Co>Cu>Li. Presence of these phytoconstituents and essential trace elements makes the Swertia species important from medicinal point of view. These are used as anti-inflammatory, hypogycaemic, hepatoprotective, antibacterial and antipyretic agent. Alcoholic extract of the plants of this genus have shown cardiovascular, CNS depressant, tuberculostatic, antipyretic, anti - diabetic activities. Swertia chirata is also diuretic and possess antimicrobial properties. Due to its medicinal properties Swertia chirata has been listed in Indian Pharmaceutical codex, British and American pharmacopoeias.

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However, a matter of concern is that these plants are mostly collected from wild as they are not cultivated. Due to its high medicinal value it has been collected extensively. This has brought some of the important species of this genus on the verge of extinction. It has been listed as critically endangered in India and vulnerable in Nepal by CITES and IUCN.

Considering the medicinal importance of Swertia species, our current study was undertaken to look at the phytochemistry of one of the species of Swertia, i.e Swertia cuneata. One of the constituent from Swertia cuneata was isolated for the first time. We also studied the bioactivity of the constituent identified through the available literature.

MATERIALS AND METHODS

Plant Collection and Identification

The plant Swertia cuneata was collected from Pindari glacier in Kumaun Himalayas at an altitude of 3353 m above sea level and was identified by the Botany Department of D.S.B. Campus, Kumaun University, Nainital.

Swertia cuneata

Extraction

The fine powdered plant material was extracted in a soxhlet apparatus with aqueous ethanol/methanol for about 36 hours. The extract was cooled, filtered and concentrated under reduced pressure till a residual mass was obtained. The residual mass was then partitioned with chloroform: water (50:50, v/v) for about 8 hours on a water bath. Each layer was separated by a separating funnel and concentrated under reduced pressure. Chloroform layer was further refluxed with n – hexane / ether (60-80°) and water fraction first with ethyl acetate and then with n – butanol.


Soxhlet Apparatus

Chromatographic Separation

Various chromatographic methods were used to check best separation of each fraction.

A. Paper Chromatography

Whatman no. 1 or 3 mm paper sheet was used to study all the extracts. Whatman no. 1 was used for analytical while no. 3 for preparative purposes.

B. Thin Layer Chromatography

Thin layer chromatography plates were prepared by applying a slurry of silica gel G (E Merck) in water on glass plates by using a spreader or manually with the help of glass rod to get homogeneous layer.

C. Column Chromatography

A glass column was first washed and then dried. It was then packed with slurry of silica gel either in n-hexane or appropriate solvent petroleum ether. The column was left overnight to allow the adsorbent to settle.

The experimental solution was adsorbed on the top of the column and it was eluted with various solvent systems starting from non – polar to polar. The ratio of adsorbent to water was (1: 2.v/v). The plates were dried at room temperature and activated by heating at 105° C for 30 minutes in an oven.

Experimental solutions were applied on the plates by the help of a thin capillary tube and spots were dried with a hot air blower. These spotted plates were developed in a variety of solvent system and dried at room temperature.

The plates were examined in visible as well as

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under long range UV light. These plates were then sprayed with different spraying reagents.

D. HPLC (High Performance Liquid Chromatography)

The normal and reverse phase high performance liquid chromatography was used for the separation and purification of various compounds.

The filtered mixture was injected through U6 K universal injector. Normal and reverse phase cartidgen were used for best separation. The variable wave length UV detector was monitored from 190 – 700 nm to achieve best resolution at a particular wave length and flow rate was adjusted in b/w 1ml per minute to 3.5ml per minute.

RESULT AND DISCUSSION

The yellow coloured compound A obtained from the sillica gel G CC of defatted chloroform fraction was repeatedly purified by TLC and HPLC methods.

On the basis of microelemental analysis results and mass spectrometry its molecular formula was assigned $C_{15}H_{12}O_6$. MS, mm/z 288(M^+), m.p. 190-191°. It fluorescened brown in UV light (365 nm), gave green colour with iron III chloride and dark brown on exposure to I_2 vapours. These colour reactions coupled with the pattern of UV spectra (λ^{MeOH}) max nm, 224, 248, 304 and IR (KBr pellets)ʊ cm^{-1}, 3350(OH), 1650, 1651(C=O) 1160and 1060 suggested it to be a xanthone.

Compound A gave positive tests for hydroxyl groups it was treated with dimethyl sulphate and potassium carbonate, 1,3,5,8- tetramethoxyxanthone 8m/z 316 (M^+), m.p. 209° was formed. In the parent xanthone the enhancement of the mass unit by 28 was indicative of two OH groups. This reaction coupled with the above spectral results showed the oxygenation pattern in the xanthone as 1, 3, 5, 8.

It formed a complex with $AlCl_3$ as evidenced by a bathochromic shift in UV spectra but it was stable to HCl indicating the presence of a chelated OH group peri to to carbonyl group but absence of ortho dihydroxy groups. The presence of chelated OH group and absence of ortho dihydroxy group was further supported by by the low field ^{1}H NMR signal at 13,12.

The two sets of proton signals at 7.32, 7.14 (each d, J = 8, 5Hz) and 6.33, 6.30 (each d, J=2, 5Mz) were assigned for ortho and meta coupled protons respectively. The chelated hydroxyl proton is either at C–1or C-8 as the low field signal for C-1 or C-8 which appears in the lower region 8-8.5 was absent. The ^{13}C NMR carbonyl signal at 180.60 clearly showed that the peri positions to carbonyl group are occupied by OH and OCH_3, groups and not both by OH or OCH_3, groups. (Hostettmann and Miura). In the event of C-1 and C-8 occupied by OH groups carbonyl carbon have been appeared down field approximately by 4 ppm whereas when occupied by OCH_3, upfield approximately by 6 ppm (Hostettman and Miura). Further, the deshielded ^{1}H NMR signal for OCH_3 group is at C-8 and OH at C-1 (Hostettmann et al.,1977). The significant mass fragments appearing at 271 (M-OH), 270 (M-H_2O) and 259 (M-CHO) were also in accordance with OCH_3 group at C-8 (Hostettmann, Miura and Arends et al.,).

The above structure of the compound was further confirmed by its chemical transformations.

Methylation

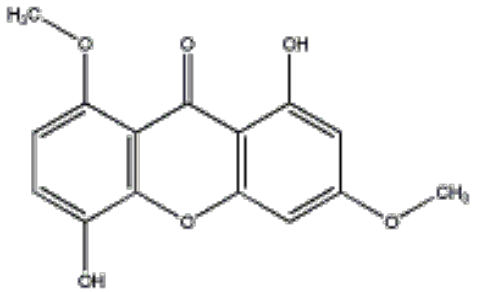
On methylation with dimethyl sulphate and potassium carbonate, compound A Yielded B (1,3,5,8-tetramethoxyxanthone).

Melting point - 209°

Molecular formula – $C_{17}H_{16}O_6$

Mass spectra – m/z 316 (H^+)

Formation of 1,3,5,8-tetramethoxyxanthone confirmed the structure of compound A as 1,5-dihydroxy 3,8-dimethoxyxanthone.



Compound A

Compound **A** shows non acidic character. The position of second OCH_3 at C-3 and OH at C-5. Since one of the hydroxyl groups peri to carbonyl frequencies is free and chelated, the protons of the same ring absorb at higher field than non chelated one (Hostettmann et al.). the meta coupled proton signal at 6.30 and 6.33 were assigned to C-2 and C-4 and the

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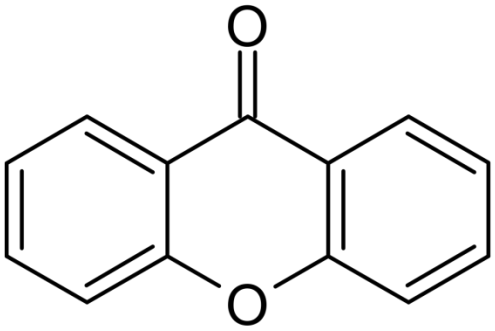
ortho coupled proton at 7.32 and 7.14 to C-6 and C-7, respectively.

^{13}C NMR spectra of the compound A showed 15 carbon signals. The low field signal at 180.60 (δ) belonged to C=O and two quartets at 62.8 and 55.8 were due to two methoxyl carbons. Out of the left over twelve carbon signals six low field signals were due to six oxygenated carbon atoms belonging to C – 1, C – 3, C – 4a, C – 4b, C – 5 and C – 8. The remaining four methyl carbon signals were assigned to C – 2, C – 4, C – 6 and C – 7.

All the above chemical and spectral data indicate that compound A was identified as 1,5,dihydroxy-3,8-dimethoxyxanthone, a new xanthone isolated for the first time from Swertia cuneata.

Bioactivity of Xanthones



Xanthone

Xanthonoids are the major class of compounds among the phytoconstituents of this genus, and, since they often exhibit broad spectrum effects, they have created a stir among pharmacologists and biologists. Particularly, tetra-oxygenated xanthones have been reported to exhibit hypo-glycaemic, anti-hepatotoxic, anti-malarial, anti- inflammatory, anti-oxidant, anti-microbial, and anti-tumor properties (Pant et al., 2002, Menkovic et al., 2002, Basnet et al., 1994). Extracts of Swertia chirata were found to be effective against Gram positive and Gram-negative bacteria.

The bitter principle found in the plants belonging to the family Gentianaceae is due to the presence of xanthones. These plants are therefore used in traditional remedies against loss of appetite and fever and as bitter tonic. Xanthones (especially mangiferin) are reported to give CNS stimulation and have anti-inflammatory activity. A crude extract of Swertia has been reported to display insect repellent activity. An extract from S. paniculata is used in treatment of some mental disorders. Mangiferin was the first xanthone to be investigated pharmacologically. Anti-inflammatory activity has also been observed for mangiferin. Alcoholic extract of the plant of this genus have shown cardiovascular, CNS depressant, antipyretic anti- psychotic, tuberculostatic, mutagenic as well as anti- diabetic activities. (Ghosal et al, 1975,1978, Bhattacharya et. al, 1976). Studies of magniferin on malarial parasite and fungi were done by Goyal et al.(1981). Verma et al., (2013) described the anti-hyperglycemic activity of Swertia chirayita and Andrographis paniculata plant extrtacts in streptozocin-induced diabetic rats. The mutagenic activity of 1,8-dihydroxy 3,5-dimethoxy xanthone; 1,8-dihydroxy 3,7-dimethoxy xanthone; 1-hydroxy 3,5,8-trimethoxy xanthone; 1,7,8-trihydroxy 3-methoxy xanthone; 1,5,8-trihydroxy 3-methoxy xanthone; 1,3,7,8-tetrahydroxy-xanthone; 1,3,5,8-tetra-hydroxy xanthone and 8-hydroxy 1,3,5-trimethoxy xanthone were determined by Kanamori et al. (1984). Xanthone glucosides and prenylated xanthones, mangiferin, mangostin, mangostin triacetate and isomangostin are known to possess effective anti- inflammatory activities. Balasundari et.al. investigated free radical scavenging xanthones and tumor cell growth inhibition from Swertia chirayita.

CONCLUSION

The plants of genus Swertia carry immense medicinal value. It is an ingredient many Ayurvedic formulations. The traditional use of these plants for remedial purposes ensures its positive outcomes with less or no side effects. Although Swertia is one of the most studied genus but the phytoconstituents present in these plants need more exploration so that they they might be helpful in new drug discovery and enhanced therapeutic effects. Studies carried out on the biological activities of the chemical constituents of Swertia species will help in understanding their mechanism of action. But overexploitation and unthoughtful harvesting from the wild has destroyed its habitat. Cultivation of this species can save them from being extinct. It will also help to conserve the biodiversity of this region. Cultivation of medicinal plants can also generate a source of income for the people of rural areas in this region.

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